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Abstract: Ammonium chloride addition to drinking water is often used to induce metabolic acidosis (MA) in rodents but may also cause mild dehydration. Previous microarray screening of acidotic mouse kidneys showed upregulation of genes involved in renal water handling. Thus, we compared two protocols to induce metabolic acidosis in mice and rats: standard 0.28M NH(4)Cl in drinking water or an equivalent amount of NH(4)Cl in food. Both treatments induced MA in mice and rats. In rats, NH (4)Cl in water caused signs of dehydration, reduced mRNA abundance of the vasopression receptor 2 (V2R), increased protein abundance of the aquaporin water channels AQP2 and AQP3 and stimulated phosphorylation of AQP2 at residues Ser256 and Ser261. In contrast, NH(4)Cl in food induced massive diuresis, decreased mRNA levels of V2R, AQP2, and AQP3, did not affect protein abundance of AQP2 and AQP3, and stimulated phosphorylation at Ser261 but not pSer256 of AQP2. In mice, NH(4)Cl in drinking water stimulated urine concentration, increased AQP2 and V2R mRNA levels, and enhanced AQP2 and AQP3 protein expression with higher levels of AQP2 pSer256 and pSer261. Addition of NH(4)Cl to food, stimulated diuresis, had no effect on mRNA levels of AQP2, AQP3, and V2R, and enhanced only AQP3 protein abundance whereas pSer256-AQP2 and pSer261-AQP2 remained unaltered. Similarly, AQP2 staining was more intense and luminal in kidney from mice with NH(4)Cl in water but not in food. Pendrin, SNAT3 and PEPCK mRNA expression in mouse kidney were not affected by the route of N(4)Cl application. Thus, addition of NH(4)Cl to water or food causes MA but has differential effects on diuresis and expression of mRNAs and proteins related to renal water handling. Moreover, mice and rats respond differently to NH(4)Cl loading, and increased water intake and diuresis may be a compensatory mechanism during MA. It may be necessary to consider these effects in planning and interpreting experiments of NH(4)Cl supplementation to animals.

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Induction of metabolic acidosis with ammonium chloride (NH_4Cl) in mice and rats – species differences and technical considerations

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ABSTRACT

Ammonium chloride addition to drinking water is often used to induce metabolic acidosis (MA) in rodents but may also cause mild dehydration. Previous microarray screening of acidotic mouse kidneys showed upregulation of genes involved in renal water handling. Thus, we compared two protocols to induce metabolic acidosis in mice and rats: standard 0.28M NH_4Cl in drinking water or an equivalent amount of NH_4Cl in food. Both treatments induced MA in mice and rats. In rats, NH_4Cl in water caused signs of dehydration, reduced mRNA abundance of the vasopression receptor 2 (V2R), increased protein abundance of the aquaporin water channels AQP2 and AQP3 and stimulated phosphorylation of AQP2 at residues Ser256 and Ser261. In contrast, NH_4Cl in food induced massive diuresis, decreased mRNA levels of V2R, AQP2, and AQP3, did not affect protein abundance of AQP2 and AQP3, and stimulated phosphorylation at Ser261 but not pSer256 of AQP2. In mice, NH_4Cl in drinking water stimulated urine concentration, increased AQP2 and V2R mRNA levels, and enhanced AQP2 and AQP3 protein expression with higher levels of AQP2 pSer256 and pSer261. Addition of NH_4Cl to food, stimulated diuresis, had no effect on mRNA levels of AQP2, AQP3, and V2R, and enhanced only AQP3 protein abundance whereas pSer256-AQP2 and pSer261-AQP2 remained unaltered. Similarly, AQP2 staining was more intense and luminal in kidney from mice with NH_4Cl in water but not in food. Pendrin, SNAT3 and PEPCK mRNA

expression in mouse kidney were not affected by the route of NH_4Cl application. Thus, addition of NH_4Cl to water or food causes MA but has differential effects on diuresis and expression of mRNAs and proteins related to renal water handling. Moreover, mice and rats respond differently to NH_4Cl loading, and increased water intake and diuresis may be a compensatory mechanism during MA. Thus, it may be necessary to consider these effects in planning and interpreting experiments of NH_4Cl supplementation to animals

keywords: Ammonium chloride, metabolic acidosis, rodents, aquaporins, pendrin, ammoniagenesis

INTRODUCTION

The kidney plays a central role in maintaining the composition of body fluids by regulating water, NaCl, acid-base, and solute reabsorption and excretion, respectively. The role of specific genes in these processes have been studied extensively over the last decade using genetically modified animals, often requiring provocation tests to uncover defects. The standard protocol to induce metabolic acidosis in experimental animals involves application of ammonium chloride (NH_4Cl), which is supplied most frequently in drinking water [1-11], but also in food or fixed amounts of gelled diet [6, 12-16]. Administration of NH_4Cl in both, drinking water and food is also used [17-18].The first report describing the effect of ammonium chloride ingestion on acid-base balance dates back to 1921 [19].

Metabolic acidosis induced by NH_4Cl administered in food and drinking solution has been associated with decreased reabsorption of NaCl and water in the proximal tubule of dog and rat [17, 20-21]. In the collecting duct in rats, MA (NH_4Cl provided in gelled diet or standard 0.28M NH_4Cl solution) has been linked to altered expression and activity of the epithelial sodium channel ENaC [13, 22]. Moreover, altered expression of the vasopressin-regulated water channel AQP2 and a concomitant increase of circulating levels of vasopressin in response to 0.28M NH_4Cl in water loading have been described in rats [9]. In contrast, Nanogutchi and colleagues suggested that elevated expression of AQP2 in rats was due to dehydration and not MA itself [16]. Similarly, a study on the aquaporin

6 water channel revealed elevated abundance of AQP6 only in rats receiving NH_4Cl in water but not in food [6]. The authors suggested that changes in water intake and not disturbed acid-base status of animals would be responsible. Recently, microarray screening of kidneys from 2 and 7 days acidotic mice revealed the upregulation of a number of genes involved in water homeostasis (AQP1, AQP2, AQP3, AQP4, the vasopressin receptor V2R, and the urea transporter Slc14a2) [23]. Acidotic mice, however, consumed around 20% less water than control animals.

Thus, the effects of acidosis and particularly the application of NH_4Cl have remained controversial with respect to its effects on renal water handling. Most authors used the standard protocol, adding 0.28M NH_4Cl to drinking water [9, 21, 24-27]. Nonoguchi et al. added NH_4Cl to food [16]. Therefore discrepancies observed in these studies may be explained by different routes of NH_4Cl application, variable degrees of dehydration in studies adding NH_4Cl to drinking water, and species differences between rats and mice.

Therefore, we aimed to clarify if the route of NH_4Cl application affects the physiologic response of the kidney and examined the induction of metabolic acidosis in response to standard 0.28M NH_4Cl in drinking water and compared it with NH_4Cl addition to food. Both protocols were tested in mice and rats to test also for species specific responses. Our data indicate that NH_4Cl in drinking water induces dehydration in addition to MA and that some of the effects ascribed previously to MA may be rather the consequences of dehydration.

Moreover, distinct differences between mice and rats exist in their response to NH_4Cl-loading.

METHODS

Animals

All experiments were performed on 12 weeks old C57BL/6J male mice (25-30 g) and male Wistar rats (160-200 g). All experiments were performed according to Swiss Animal Welfare laws and approved by the local veterinary authority (Veterinäramt Zürich). All animals received standard rodent chow GLP 3433 (PROVIMI KLIBA, Kaiseraugst, Switzerland).

Induction of metabolic acidosis

Two experimental protocols were used:

***Protocol 1: Experimental protocol for acid load in drinking water*.** C57BL/6J male mice and Wistar rats were given 0.28 M NH_4Cl + 0.5% sucrose in drinking water (tap water) for 3 days. Control animals received tap water + 0.5% sucrose ad libitum. All animals had free access to standard rodent chow. This acid load averaged 6.74 ± 0.49 mmoles NH_4Cl/day/rat and 1.06 ± 0.06 mmoles NH_4Cl/day/mouse.

Protocol 2: Experimental protocol for acid load in food. C57BL/6J male mice and Wistar rats were given NH_4Cl mixed with standard powdered rodent chow for 3 days in the following proportion: 2 g (mice) or 3 g (rats) of NH_4Cl / 100 g of standard rodent chow. All animals received tap water + 0.5% sucrose ad libitum and had free access to the chow. Control animals received the same diet but without NH_4Cl. This acid load averaged 9.41 ± 0.24 mmoles NH_4Cl/day/rat and 1.38 ± 0.04 mmoles NH_4Cl/day/mice.

Metabolic cages studies

All animals were adapted to metabolic cages for 3 days before data collection. In total, 4 rats and 6 mice were acid-loaded using *Protocol 1*, 4 rats and 6 mice were acid-loaded using *Protocol 2*, and 4 rats and 6 mice served as controls. Daily chow, water intake and body weights were measured and urine was collected under mineral oil. At the end of experiments, animals were anesthetized with isoflurane/pressurized air and heparinised arterial blood was collected from the tail artery in rats and heparinised mixed venous blood was collected in mice and analyzed immediately for pH, blood gases and electrolytes on a Radiometer ABL 800 (Radiometer, Copenhagen, Denmark) blood gas analyzer. Serum was collected and frozen until further analysis. Both kidneys were harvested, rapidly frozen in liquid nitrogen, and stored at -80°C until mRNA extraction.

Urine and serum analysis was performed as described previously [23]. Aldosterone in urine was measured with a radioimmunoassay using a commercially available kit (DPC Dade Behring, La Défense, France). Briefly, 24 hour urines were collected in metabolic cages and kept at -20°C until use. For each sample, 50 µl urine were hydrolized overnight with 3.2 N HCl, treated with ethyl acetate to extract aldosterone and evaporated. Dried extracts were resuspended in the appropriate solvent following the manufacturer's recommendations and aldosterone was then measured by radioimmunoassay.

RNA extraction, reverse transcription, and real-time quantitative PCR

Kidneys were harvested and rapidly frozen in liquid nitrogen. Total snap-frozen kidneys were homogenized in RLT-Buffer/1% ß-mercaptoethanol (Qiagen, Basel, Switzerland). Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Quality and concentration of the isolated RNA preparations were analyzed by the ND-1000 spectrophotometer (NanoDrop Technologies). 300 ng of RNA was used as a template for reverse transcription using the TaqMan Reverse Transcription Kit (Applied Biosystems, Forster City, CA, USA). Real time RT-PCR was performed as described previously [23].

Western Blot Analysis

Preparation of crude membrane proteins from the total kidneys was performed as described elsewhere [26]. 50 µg of crude membrane proteins were solubilised in Laemmli sample buffer and SDS-PAGE was performed on 10 % polyacrylamide gels. Immunoblotting and image analysis was performed as described previously [23, 28]. The primary antibodies used were: rabbit anti aquaporin 2 (1:5000) (kindly provided by J. Loffing, Institute of Anatomy, University of Zurich, Switzerland [29], rabbit anti aquaporin 3 (1:200) (Alomone Labs, Jerusalem, Israel), rabbit anti pSer256-AQP2 1:3000 (kindly provided by Dr. Soren Nielsen, University of Aarhus, Denmark), rabbit anti pSer261-AQP2, 1:2000 (Lifespan Biosciences, Seattle, WA, USA), and mouse monoclonal anti-β-

actin antibody (42 kD; Sigma, St. Louis, MO; 1:5000); secondary antibodies used were: donkey anti-rabbit or sheep anti-mouse antibodies linked to horseradish peroxidase (1:10000) (GE Healthcare, Little Chalfont Buckinghamshire, UK) or goat anti-rabbit antibody (1:5000) linked to alkaline phosphatase (Promega, USA).

Immunohistochemistry

Mouse kidneys were perfusion fixed through the right ventricle with a PFA fixative solution and subsequent immunohistochemistry was performed as described previously [25, 30]. Sections were incubated with polyclonal goat anti-human aquaporin 2 (1:1000) (Santa Cruz Biotechnology) followed by Alexa 488-conjugated donkey anti-goat antibody (1:400) (Invitrogen, Basel, Switzerland).

Statistical analysis

Results are expressed as mean ± SEM. All data were tested for significance using the one way ANOVA and Tukey post test or unpaired student's t-test where appropriate.

RESULTS

NH_4Cl in water induces metabolic acidosis and dehydration in rats

In rats acid-loading with NH_4Cl either in drinking water or food resulted in induction of metabolic acidosis, as evident from the reduction of blood pH and bicarbonate levels (Table 2). However, the degree of metabolic acidosis was less pronounced in rats ingesting NH_4Cl with food despite ingesting even a slightly higher load (6.74 ± 0.49 mmoles NH_4Cl/day/rat. for *Protocol 1*; 9.41 ± 0.24 mmoles NH_4Cl/day/rat for *Protocol 2*). As expected serum chloride was elevated in both acidotic rat groups, however, animals ingesting NH_4Cl in food had significantly lower chloride levels than animals with NH_4Cl in water. Plasma sodium concentration and hematocrit were not changed in both experimental rat groups (Table 2). Plasma urea concentration and osmolality were elevated only in rats NH_4Cl-loaded with water (Table 2). During acid-loading rats gained less weight when receiving NH_4Cl in drinking water and their food intake was lower compared to control (Table 2). Water consumption increased significantly in the group receiving NH_4Cl in food paralleled by higher urinary output.

Urine analysis revealed that rats from both acidotic groups acidified their urine to a similar extend and ammonium excretion as well as titratable acid (TA) and net acid excretion (NAE) was increased accordingly. NH_4Cl-loading in water did not influence water intake and urine output but increased urine osmolality. NH_4Cl-loading in food, however increased urine volume without changing urine osmolality. Urinary urea and aldosterone were elevated in both acidotic rat

groups, with a 3-fold higher aldosterone in animals receiving NH_4Cl in water than in food (Table 2).

Changes in AQP2, AQP3, V2R, and αENaC mRNA and protein expression in rat kidney depend on the route of NH_4Cl application.

Induction of MA for 3 days with 0.28M NH_4Cl in the drinking water in rats had no effect on mRNA expression of both AQP2 and AQP3, and α subunit of epithelial sodium channel αENaC but decreased V2R mRNA expression (Figure 1). In contrast, addition of NH_4Cl to food decreased mRNA expression of both aquaporins, V2R, and αENaC (Figure 1).

Induction of metabolic acidosis using NH_4Cl-containing drinking solution resulted in elevated abundance of both, glycosylated and non-glycosylated AQP2 species and AQP3 in rat kidney (Figure 2A,B). Application of NH_4Cl in food had no apparent effect on AQP2 and AQP3 protein abundance (Figure 2A,B). To estimate the impact of NH_4Cl application on AQP2 activation, we assessed phosphorylation of AQP2 at positions Ser256 and Ser261 (Figure 3A,B). Phosphorylation of Ser256 is associated with activation of AQP2 [31-32], whereas phosphorylation of Ser261 may be associated with increased internalization and degradation [33-34]. Addition of NH_4Cl to drinking water stimulated phosphorylation at serines 256 and 261 in native rat kidney. In contrast, NH_4Cl in food, stimulated only phosphorylation at serine 261 (Figure 3A,B)

NH_4Cl-loading alters distribution of AQP2 water channel

Immunohistochemistry was used to examine whether NH_4Cl-loading alters segmental and/or subcellular distribution of the aquaporin 2 water channel in rat kidney. AQP2 is predominantly expressed on the apical side of principal cells along the entire connecting tubule and collecting duct system [35]. Low magnification overviews showed a higher intensity for AQP2 staining in kidneys from animals receiving NH_4Cl in water but not in food (Figure 4 A). At higher magnification a more pronounced membrane associated staining in CNT, CCD, OMCD, and IMCD was seen in all NH_4Cl treated rats which appeared to be even more intense in rats receiving NH_4Cl in water.

NH_4Cl in water induces more pronounced metabolic acidosis and decreases diuresis in mice

In mice acid-loading with both protocols resulted in a fall of blood pH and HCO_3^- and in increases in Cl^- and Ca^{2+} levels. Neither serum urea nor osmolality were affected by acid-loading, also hematocrit was unchanged in both acidotic groups. Mice with NH_4Cl in food lost body weight, whereas mice on normal diet or receiving NH_4Cl in water did not (Table 3); however food intake was not changed by either way of acid-loading. Water consumption was elevated in mice receiving NH_4Cl in food paralleled by higher urinary output.

Acid loading with both protocols resulted in stimulated urinary acidification,elevated ammonium, titratable acids, and net acid excretion. Interestingly, mice ingesting NH_4Cl in food showed much higher urinary

ammonium excretion than NH_4Cl-loaded in water, although NH_4Cl intake was not significantly different between protocols (1.19 ± 0.08 mmoles NH_4Cl/day/mouse for NH_4Cl in water; 1.38 ± 0.04 mmoles NH_4Cl/day/mice for NH_4Cl in food). Elevated urine osmolality in mice acid-loaded in water was paralleled by lower urine output despite normal water intake. NH_4Cl-loading in food, however, increased water consumption and urine volume and reduced urine osmolality. Both NH_4Cl loading protocols increased urinary aldosterone excretion to a similar extent.

NH_4Cl in water increases AQP2 and AQP3 mRNA and protein abundance

In mice acid-loading with NH_4Cl in water resulted in increased AQP2 and V2R mRNA abundance but had no effect on expression of AQP3 and αENaC mRNA (Figure 5). In contrast to rats, mice ingesting NH_4Cl in food did not show any changes in mRNA abundance of AQP2, AQP3, or V2R, but expression of αENaC mRNA was elevated (Figure 5). Furthermore, only mice ingesting NH_4Cl in water but not in food had elevated abundance of glycosylated and non-glycosylated AQP2 (Figure 6A,B). Moreover, both acidotic mice groups exhibited elevated levels of the aldosterone regulated AQP3 water channel (Figure 6A,B). Addition of NH_4Cl to drinking water stimulated AQP2 phosphorylation at serines 256 and 261 in native mouse kidney. In contrast, NH_4Cl in food had no significant effect on AQP2 phosphorylation at serine residues 256 and 261 (Figure 7A,B).

NH_4Cl-loading alters distribution of AQP2 water channel

Immunohistochemistry of mouse kidneys showed similar findings as in rat kidney. AQP2 staining in CNT, CCD, OMCD and IMCD appeared to be more membrane associated in mice treated with NH_4Cl in water but also mice with NH_4Cl in water had some AQP2 at the luminal membrane (Figure 8A,B).

NH_4Cl in food and water alters abundance of acidosis regulated genes

We and others have previously shown that the anion exchanger pendrin, the glutamine transporter SNAT3, and phospho-enol pyruvate carboxy kinase (PEPCK) are regulated on mRNA levels during metabolic acidosis induced by NH_4Cl [7, 10, 23, 36, 37]. qPCR demonstrated that pendrin mRNA abundance was similarly reduced in mice receiving NH_4Cl in food or drinking water (Figure 5). Also, SNAT3 and PEPCK mRNA levels were increased in both mouse groups irrespective of the route of NH_4Cl administration (Figure 5).

DISCUSSION

The purpose of this study was to compare the induction of metabolic acidosis with NH_4Cl added either to drinking water or food. Application of NH_4Cl with drinking water is a well established and extensively used method to induce metabolic acidosis [1-4, 7-8, 10, 27]. In our hands, both protocols induced MA in rats and mice as evident from lower blood pH, increased urinary acidification, and acid excretion. Both acidotic mice and rat groups were also able to acidify their urine and excrete acids to a similar extend regardless of the diet (Tables 2-3). It seems, however, that animals receiving NH_4Cl in food were less acidotic compared to the group receiving NH_4Cl in drinking water. Total NH_4Cl intake was similar in both groups suggesting that the route of application affects compensatory mechanisms.

The major findings of our comparison of different NH_4Cl-loading protocols are: 1) both protocols induced metabolic acidosis in mice and rats, 2) differences between rats and mice exist with respect to their renal compensatory responses as evident from different expression levels of AQP2, AQP3, αENaC, and V2R mRNA and protein, 3) NH_4Cl in drinking water induces signs of a mild dehydration in rats, as demonstrated by higher aldosterone levels and higher urine osmolality, 4) NH_4Cl in water stimulated urinary concentration in mice without other signs of dehydration, 5) NH_4Cl in water increased phosphorylation of residues in AQP2 associated with higher AQP2 activity and insertion into the membrane paralleled by enhanced staining of AQP2 at the luminal membrane,

and 6) dehydration and MA affect independently expression patterns of genes and proteins involved in renal water handling.

We found higher urea excretion in rats and mice in the food group as compared to the water group. In contrast, ammonium excretion was higher only in mice receiving NH_4Cl in food compared to mice with NH_4Cl in water. Mice and rats on the acid diet tended to receive a higher NH_4Cl load than the respective water groups and therefore an overall increased nitrogen load that might explain the observation. Moreover, the higher urinary urea excretion might also reflect, at least in mice, increased catabolism of proteins as suggested by a higher weight loss.

The excretion of the chloride load (given by way of the chloride contained in food plus the NH_4Cl added either drinking water or food) was lower in rats receiving NH_4Cl in food. Since urinary chloride excretion might reflect net chloride intake and ammonium absorption, it might indicate a lower absorbed NH_4Cl load in rats receiving NH_4Cl in food. However, interpretation of urinary chloride excretion is difficult since it may not directly correlate with intestinal chloride or even ammonium absorption. Recent experiments investigating sodium balance in rats and mice demonstrated that intake and excretion did not equal and that substantial amounts of sodium were stored under the skin [38]. Thus, more detailed studies on the net absorptive rate of ammonium intake are needed to estimate the true acid load provided by the two different application routes.

Dehydration and extracellular volume contraction activates the renin-angiotensin-aldosterone system and stimulates synthesis of angiotensin II and

secretion of aldosterone [39-42]. Induction of MA resulted in elevation of urine aldosterone excretion in both acidotic rat and mice groups; however, aldosterone levels were 3-fold lower in rats receiving NH_4Cl in food compared to the NH_4Cl in water group. Surprisingly, elevated aldosterone levels were not reflected by changes in urinary sodium and potassium excretion. This might be explained by direct effects of acidosis on aldosterone targets such as the ROMK potassium channel or the ENaC sodium channel. The function of ROMK is modulated by intracellular pH where a fall in intracellular pH reduces channel function and potassium secretion [43-44]. Water reabsorption by the luminal AQP2 water channel in the collecting duct is a major factor in water homeostasis and urinary concentration. The subcellular localization and activity of this channel is regulated on the molecular level by phosphorylation of several residues including serines 256 and 261[31-34] integrating various signals including stimulation by vasopressin or angiotensin II [45]. Our results indicate that AQP2 is stimulated as indicated by increased phosphorylation of Ser256 and by enhanced staining at the luminal membrane in animals receiving NH_4Cl in water. Hematocrit and plasma sodium concentration were unchanged in both rat groups. Additionally NH_4Cl ingestion in food resulted in increased water intake and urine output and decreased urine osmolality in both mice and rats which is in agreement with observations of Mori et al. [15]. Collectively, these data are consistent with mild dehydration in animals receiving NH_4Cl in drinking water resulting in elevated aldosterone levels and upregulation and stimulation of several components of the urinary concentration machinery. The regulation of AQP3 observed also in the

NH_4Cl in food group may be at least in part due to the slightly increased levels of aldosterone which has been shown to increase AQP3 expression [46].

All animals receiving NH_4Cl in food or water developed increased urinary calcium excretion which was more profound in the food groups. Hypercalciuria is thought to develop at least in part due to inhibition of the TRPV5 calcium channel in the distal convoluted tubule and connecting tubule [11]. Elevated urinary calcium may then affect localization and expression of the AQP2 water channel along the collecting duct via a calcium-sensing receptor [47-48] promoting increased diuresis. However, whether this mechanism contributes to the higher urinary excretion in the food group remains to be examined.

Our results of NH_4Cl-loading in drinking water are similar to those of Amlal et al. [9] suggesting that the results reported there may be mainly due to dehydration but not MA in rats. Ingestion of equivalent amounts of NH_4Cl in food had no effect on AQP2 protein levels in our hands. Also immunohistochemistry analysis of mouse and rat kidneys showed more intense AQP2 staining with enhanced accumulation at the luminal membrane more pronounced in the NH_4Cl in water group. Concomitant with increased protein abundance was mRNA expression of AQP2 in mice loaded with NH_4Cl in water. Interestingly, there was no change in AQP2 mRNA expression in rats subjected to NH_4Cl in water. Moreover, expression of AQP2 was even decreased in animals ingesting NH_4Cl in food. Thus, not urinary concentration but increased diuresis may actually be part of the renal compensatory response to metabolic acidosis.

Taken together, our study demonstrates that the route of NH_4Cl application – drinking water versus food – can affect the regulation of renal genes and proteins, particularly those involved in water handling. NH_4Cl in drinking water causes mild dehydration and upregulation and stimulation of several components of the urinary concentrative mechanism. Moreover, our study shows that distinct species differences exist between mice and rats in their response to NH_4Cl-loading in drinking water. Therefore, future studies should consider these differential effects of NH_4Cl addition in planning and interpreting animal experiments. In addition, species differences exist between rats and mice and may be more pronounced than previously anticipated. Finally, it may be of interest to consider similar studies in humans assessing urinary concentration and water balance.

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Table 1. Primers and probes used for real-time PCR

Gene	Acc. No.	Primers	Probe
AQP2 (M)	NM_009699	F: 5'-TGGTGCTGTGCATCTTTGCCT-3' (503-523) R: 5'-ACTTGCCAGTGACAACTGCTG-3' (655-673)	5'-ACCTCCTTGGGATCTATTTCACCGG-3' (596-620)
AQP2 (R)	NM_012909	F: 5'-TGGTGCTGTGCATCTTTGCCT-3' (506-526) R: 5'-ACTTGCCAGTGACAACTGCTG-3' (658-676)	5'-ACCTCCTTGGGATCTATTTCACCGG-3' (599-623)
AQP3 (M)	NM_016689	F: 5'-TCAGAAGTCTTCACGACTGGCC-3' (763-784) R: 5'-ATGTGGGCCAGCTTCACATTC-3' (903-923)	5'-TGGTGTCTTCGTGTACCAGCTCATG-3' (834-858)
AQP3 (R)	NM_031703	F: 5'-AGAAGTCTTTACGACTGGCCAG-3' (775-796) R: 5'-ATGTGGGCCAGCTTCACATTC-3' (913-933)	5'-TGGTGTCTTCGTGTACCAGCTCATG-3' (844-868)
V2R (M)	NM_019404	F: 5'-CGTGGGATCCAGAAGCTCC-3' (1026-1044) R: 5'-AGGCTACGCAACTCCGAGG-3' (1140-1158)	5'-CCTTTGTGTTGCTCATGCTGCTGGCTAG-3' (1059-1086)
V2R (R)	NM_019136	F: 5'-CGTGGGATCCAGAAGCTCC-3' (884-902) R: 5'-AGGCTACGCAACTCCGAGG-3' (998-1016)	5'-CCTTTGTGTTGCTCATGCTGCTGGCTAG-3' (917-944)
αENaC (M)	NM_011324	F: 5'-GGTGCACGGTCAGGATGAG-3' (1681-1699) R: 5'-TAGTTGCCTCCGAGGCTGTC-3' (1778-1797)	5'-CTGCTTTTATGGATGATGGTGGCTTCAA-3' (1701-1728)

αENaC (R)	NM_031548	F: 5'-TGCCGGAAGCCTTGTAGTGTGA-3' (1872-1893) R: 5'-AAGATCCAATCCTGGGACTTCA-3' (1931-1957)	5'-CAACTACAAACTCTCTGCCGGCTACTCACG-3' (1895-1924)
HPRT (M)	NM_013556	F: 5'-TTATCAGACTGAAGAGCTACTGTAAGATC-3' (442-471) R: 5'-TTACCAGTGTCAATTATATCTTCAACAATC-3' (539-568)	5'-TGAGAGATCATCTCCACCAATAACTTTTATGTCCC-3' (481-515)
HPRT (R)	NM_012583	F: 5'-GCTGAAGATTTGGAAAAGGTGTTTA-3' (151-175) R: 5'-ACACAGAGGGCCACAATGTGA-3' (246-266)	5'-TTATGGACAGGACTGAAAGACTTGCTCGAGATG-3' (191-223)

Table 2: NH_4Cl loading of rats

	Control	NH_4Cl in water	NH_4Cl in food
Blood			
pH	7.41 ± 0.02	7.16 ± 0.02 ***	7.25 ± 0.02 *** #
pCO_2 mmHg	41.4 ± 3.0	49.9 ± 2.6	49.7 ± 1.7
$[HCO_3^-]$ mM	25.4 ± 1.1	17.2 ± 1.0 **	20.9 ± 1.2 *
$[Na^+]$ mM	135.0 ± 2.3	139.5 ± 0.9	138.8 ± 0.3
$[K^+]$ mM	5.1 ± 0.1	4.5 ± 0.2 *	4.4 ± 0.1 *
$[Cl^-]$ mM	99.8 ± 0.9	112.0 ± 0.9 ***	105.3 ± 0.9 ** ##
$[P_i]$ mM	0.72 ± 0.02	0.85 ± 0.06	0.90 ± 0.08
$[Ca^{2+}]$ mM	1.02 ± 0.14	1.49 ± 0.04 **	1.22 ± 0.04 ##
Hematocrit %	41.7 ± 0.9	43.7 ± 0.8	43.1 ± 0.8
Urea (mg/dl)	27.2 ± 3.8	54.9 ± 2.0 ***	25.3 ± 1.2 ###
Osmolality (mOsm/kg H_2O)	294.5 ± 0.3	302.0 ± 2.9 *	284.5 ± 2.2 ##
Urine			
pH	8.67 ± 0.05	5.73 ± 0.06 ***	5.79 ± 0.04 ***
Creatinine (mg/dl)	30.4 ± 4.9	43.4 ± 3.9	13.7 ± 1.6 * ###
NH_3/NH_4^+/(mM)/crea (mg/dl)	2.45 ± 0.19	7.45 ± 0.58 ***	5.97 ± 0.73 **
TA (mEq)/crea (mg/dl)	-3.81 ± 0.69	2.06 ± 0.29	2.75 ± 0.44
NAE (mEq)/crea (mg/dl)	-1.36 ± 0.69	9.52 ± 0.86	8.72 ± 0.95
Na^+ (mM)/crea (mg/dl)	1.13 ± 0.25	1.63 ± 0.23	2.94 ± 0.42 ** ##
K^+ (mM)/crea (mg/dl)	2.69 ± 0.56	3.61 ± 0.30	5.95 ± 0.74 ** #
Ca^{2+} (mM)/crea (mg/dl)	0.091 ± 0.022	0.193 ± 0.042	0.305 ± 0.036 **
Mg^{2+} (mM)/crea (mg/dl)	0.081 ± 0.027	0.430 ± 0.053 **	0.635 ± 0.092 ***
Cl^- (mM)/crea (mg/dl)	2.14 ± 0.72	13.38 ± 2.01 **	13.25 ± 1.59 **
P_i (mM)/crea (mg/dl)	0	0.20 ± 0.02 ***	0.33 ± 0.04 *** #
24 h Aldosterone (nM)	0.008 ± 0.003	0.199 ± 0.030 ***	0.047 ± 0.005 *** ###
Urea (mg/dl)/crea (mg/dl)	40.3 ± 4.3	74.4 ± 6.0 *	100.5 ± 9.9 ***
Osmolality (mOsm/kg H_2O)	563 ± 100	1783 ± 124 ***	820 ± 47 ###
Urine volume (ml)/g BW	0.069 ± 0.014	0.045 ± 0.007	0.107 ± 0.005 * ##
Water intake (ml)/g BW	0.18 ± 0.02	0.14 ± 0.01	0.22 ± 0.01 ##
Food intake (g)/g BW	0.095 ± 0.005	0.072 ± 0.005 **	0.089 ± 0.002 #
Body weight (% change)	+14.6 ± 0.8	+2.6 ± 1.4 ***	+8.0 ± 1.0 **#
NH_4Cl load (mmoles/day)	n.a.	6.74 ±0.49	9.41 ± 0.24 ##
NH_4Cl load (mmoles/g BW)	n.a.	0.038 ± 0.002	0.050 ± 0.001 ##

Table 3: NH_4Cl loading of mice

	Control	NH_4Cl in water	NH_4Cl in food
Blood			
pH	7.36 ± 0.01	7.20 ± 0.06 *	7.27 ± 0.03 * *
pCO_2 mmHg	39.4 ± 0.8	38.8 ± 1.9	42.7 ± 1.8
$[HCO_3^-]$ mM	21.8 ± 0.8	14.8 ± 1.7 **	18.8 ± 0.7 *
$[Na^+]$ mM	145.0 ± 0.4	148.0 ± 1.1 *	146.3 ± 0.8
$[K^+]$ mM	4.9 ± 0.1	4.8 ± 0.1	4.7 ± 0.0
$[Cl^-]$ mM	112.8 ± 0.7	121.4 ± 1.1 **	120.6 ± 2.4 *
$[Ca^{2+}]$ mM	1.21 ± 0.01	1.29 ± 0.01*	1.33 ± 0.03 **
Hematocrit %	44.5 ± 1.0	43.5 ± 1.3	44.7 ± 1.7
Urea (mg/dl)	42.9 ± 2.1	43.0 ± 5.0	39.9 ± 4.2
Osmolality (mOsm/kg H_2O)	301.6 ± 1.7	300.2 ± 2.4	302.5 ± 2.7
Urine			
pH	6.01 ± 0.03	5.43 ± 0.04 ***	5.63 ± 0.06 ***
Creatinine (mg/dl)	35.7 ± 4.7	54.6 ± 10.8	17.1 ± 1.3 ** ##
NH_3/NH_4^+/ (mM)/crea (mg/dl)	0.66 ± 0.05	8.78 ± 1.12 ***	16.15 ± 1.10 *** ###
TA (mEq)/crea (mg/dl)	1.02 ± 0.03	1.76 ± 0.11 **	1.19 ± 0.17 #
NAE (mEq)/crea (mg/dl)	1.59 ± 0.05	9.43 ± 0.64 ***	12.34 ± 1.24 ***
Na^+ (mM)/crea (mg/dl)	3.86 ± 0.29	3.88 ± 0.47	6.04 ± 0.49 ** ##
K^+ (mM)/crea (mg/dl)	12.89 ± 0.56	11.05 ± 1.03	16.51 ± 0.89 * ##
Ca^{2+} (mM)/crea (mg/dl)	0.043 ± 0.007	0.080 ± 0.018	0.236 ± 0.017 *** ###
Mg^{2+} (mM)/crea (mg/dl)	0.50 ± 0.06	0.63 ± 0.09	1.09 ± 0.1 *** ##
Cl^- (mM)/crea (mg/dl)	7.58 ± 0.44	22.42 ± 2.64 ***	30.97 ± 1.97 *** #
P_i (mM)/crea (mg/dl)	1.66 ± 0.13	1.39 ± 0.21	2.37 ± 0.23 * ##
24 h Aldosterone (nM)	0.022 ± 0.001	0.031 ± 0.002 **	0.027 ± 0.001 *
Urea (mg/dl)/crea (mg/dl)	205.9 ± 20.5	161.8 ± 23.5	329.2 ± 14.8 ** ###
Osmolality (mOsm/kg H_2O)	2565 ± 316	4360 ± 543 *	2100 ± 158 ##
Urine volume (ml)/g BW	0.048 ± 0.007	0.053 ± 0.009	0.109 ± 0.007 * ##
Water intake (ml)/g BW	0.157 ± 0.013	0.158 ± 0.012	0.233 ± 0.016 *
Food intake (g)/g BW	0.154 ± 0.006	0.154 ± 0.006	0.153 ± 0.002
Body weight (% change)	0.99 ± 0.80	1.16 ± 0.70	-4.36 ± 1.08 ** ##
NH_4Cl load (mmoles/day)	n.a.	1.19 ± 0.08	1.38 ± 0.04
NH_4Cl load (mmoles/g BW)	n.a.	0.044 ± 0.003	0.057 ± 0.001

Table 4: Summary of the results

	Mice		Rats	
	NH_4Cl in water	NH_4Cl in food	NH_4Cl in water	NH_4Cl in food
Blood				
pH	↓	↓	↓	↓↓
HCO_3^-	↓	↓	↓	↓
Cl^-	↑	↑	↑↑	↑
Na^+	↑	↔	↔	↔
Osmolality	↔	↔	↑	↔
Hematocrit	↔	↔	↔	↔
Urine				
pH	↓↓	↓	↓↓	↓↓
NH_3/NH_4^-	↑	↑↑	↑	↑
TA	↑	↔	↑	↑
Urea	↔	↑	↑	↑↑
Osmolality	↑	↔	↑	↔
Aldosterone	↑	↑	↑↑↑	↑
Volume	↔	↑	↔	↑
mRNA				
AQP2	↑	↔	↔	↓
AQP3	↔	↔	↔	↓
V2R	↑	↔	↓	↓
αENaC	↔	↑	↔	↓
Protein				
AQP2 glyc.	↑	↔	↑	↔
AQP2 nonglyc.	↑	↔	↑	↔
AQP3	↑↑	↑	↑	↔
AQP2 pSer256 glyc.	↑↑	↔	↑↑	↔
AQP2 pSer261 glyc.	↑↑	↔	↑↑	↑↑

FIGURE LEGENDS

Table 1

Primers and probes used for quantitative real-time PCR in mice (M) and rats (R). All primers and probes were designed using Primer Express software (Applied Biosystems). Either primers or probes are spanning intron-exon boundaries to exclude genomic DNA contamination. The nucleotide numbers of the primers and probes are given in brackets.

Table 2

NH_4Cl-loading of rats

Blood gas status and electrolytes in blood and urine data from Wistar rats under control conditions and after 3 days acid-loading (0.28 M NH_4Cl + 0.5% sucrose in drinking water or 3g NH_4Cl / 100g food + 0.5% sucrose in drinking water). Control rats received only 0.5% sucrose in their drinking water. Values are means ± SEM, n = 4 per group. $^{*/\#}$ $p \leq 0.05$, $^{**/\#\#}$ $p \leq 0.01$, $^{***/\#\#\#}$ $p \leq 0.001$, * significantly different between control and acidotic group, $^{\#}$ significantly different between both acidotic groups.

Table 3

NH_4Cl-loading of mice

Blood gas status and electrolytes in blood and urine data from C57BL/6 mice under control conditions and after 3 days acid-loading (0.28 M NH_4Cl + 0.5% sucrose in drinking water or 2g NH_4Cl/ 100g food + 0.5 % sucrose in

drinking water). Control mice received only 0.5% sucrose in their drinking water. Values are means ± SEM, n = 6 per group. $^{*/\#}$ $p \leq 0.05$, $^{**/\#\#}$ $p \leq 0.01$, $^{***/\#\#\#}$ $p \leq 0.001$, * significantly different between control and acidotic group, $^{\#}$ significantly different between both acidotic groups.

Table 4

Short summary of findings in mice and rats using two different acid-loading protocols. ↑ - higher than in control group, ↓- lower than in control group, ↔ - not changed.

Figure 1

AQP2, AQP3,V2R, and αENaC **mRNA abundance in rat kidney.**

Real-time RT-PCR was used to assess AQP2, AQP3, V2R and αENaC mRNA levels in kidneys from control and NH_4Cl-loaded animals (n = 4 rats/group). In rat kidney, AQP2, AQP3, V2R and αENaC mRNA was reduced in animals receiving NH_4Cl in food. NH_4Cl in drinking water decreased only V2R mRNA. * $p \leq 0.05$, ** $p \leq 0.01$.

Figure 2

Expression of AQP2 and AQP3 proteins during acid-loading in rat kidney.

A) Immunoblotting for glycosylated and non-glycosylated variants of AQP2 and AQP3 water channels in total membrane fractions from control and NH_4Cl-loaded rats (n = 4 animals/group). All membranes were reprobed for β-actin. **(B)** Bar

graphs summarizing data from immunoblotting. All data were normalized against β-actin. * $p \leq 0.05$, *** $p \leq 0.001$.

Figure 3

Abundance of phosphorylated pSer256 and pSer261 AQP2 protein during acid-loading in rat kidney.

A) Immunoblotting for glycosylated and non-glycosylated variants of AQP2 water channel phosphorylated at serine residues 256 (pSer256-AQP2) or 261 (pSer261-AQP2) in total membrane fractions from control and NH_4Cl-loaded rats (n = 4 animals/group). All membranes were reprobed for β-actin. **(B)** Bar graphs summarizing data from immunoblotting. All data were normalized against β-actin. * $p \leq 0.05$, ** $p \leq 0.01$.

Figure 4

Immunolocalization of AQP2 water channel in rat kidney during acid-loading

(A) Low magnification (100 x) overview over cortex and inner medulla of rat kidneys from untreated animals (control) or animals receiving NH_4Cl in water or food, respectively. **(B)** Higher magnifications (400 x) of connecting tubule (CNT), cortical collecting duct (CCD) and inner medullary collecting ducts (IMCD).

Figure 5

AQP2, AQP3, V2R, and αENaC mRNA abundance in mouse kidney.

Real-time RT-PCR was used to assess AQP2, AQP3, V2R, and αENaC mRNA levels in kidneys from control and NH_4Cl-loaded animals (n = 6 mice/group). In mouse kidney, expression of AQP2 water channel and V2R mRNA was increased only in mice receiving NH_4Cl in water, but not in food. In contrast, the α subunit of the epithelial sodium channel ENaC was elevated only in mice receiving NH_4Cl in food. AQP3 expression remained unchanged in both groups. * $p \leq 0.05$, ** $p \leq 0.01$.

Figure 6

Expression of AQP2 and AQP3 proteins during acid-loading in mouse kidney.

A) Immunoblotting for glycosylated and non-glycosylated variants of AQP2 and AQP3 water channels in total membrane fractions from control and NH_4Cl-loaded mice. All membranes were reprobed for β-actin, 4 representative animals/ group are shown. **(B)** Bar graphs summarizing data from immunoblotting. All data were normalized against β-actin. **(C)** Immunolocalization of AQP2 water channel in kidneys from control and acid-loaded mice shows higher intensity in kidneys from mice loaded with NH_4Cl in water. Pictures were taken from the inner medulla at an original magnification of 200 x. * $p \leq 0.05$, ** $p \leq 0.01$.

Figure 7

Abundance of phosphorylated pSer256 and pSer261 AQP2 protein during acid-loading in mouse kidney.

A) Immunoblotting for glycosylated and non-glycosylated variants of AQP2 water channel phosphorylated at serine residues 256 (pSer256-AQP2) or 261 (pSer261-AQP2) in total membrane fractions from control and NH_4Cl-loaded mice (n = 4-5 animals/group). All membranes were reprobed for β-actin. **(B)** Bar graphs summarizing data from immunoblotting. All data were normalized against β-actin. ** $p \leq 0.01$, *** $p \leq 0.001$.

Figure 8

Immunolocalization of AQP2 water channel in mouse kidney during acid-loading

(A) Low magnification (100 x) overview over cortex and inner medulla of mouse kidneys from untreated animals (control) or animals receiving NH_4Cl in water or food, respectively. **(B)** Higher magnifications (400 x) of connecting tubule (CNT), cortical collecting duct (CCD) and inner medullary collecting ducts (IMCD).

Figure 9

Regulation of SNAT3, PEPCK, and pendrin in kidneys from NH_4Cl-loaded mice. Real-time RT-PCR was used to assess SNAT3, PEPCK, and pendrin mRNA levels in kidneys from control and NH_4Cl-loaded animals (n = 6

mice/group). All three mRNAs were regulated irrespective of the route of NH_4Cl application. *** $p \leq 0.001$.

REFERENCES

1 Chambrey R, Goossens D, Bourgeois S, Picard N, Bloch-Faure M, Leviel F, Geoffroy V, Cambillau M, Colin Y, Paillard M, Houillier P, Cartron JP, Eladari D: Genetic ablation of rhbg in the mouse does not impair renal ammonium excretion. Am J Physiol Renal Physiol 2005;289:F1281-1290.

2 Quentin F, Chambrey R, Trinh-Trang-Tan MM, Fysekidis M, Cambillau M, Paillard M, Aronson PS, Eladari D: The Cl^-/HCO_3^- exchanger pendrin in the rat kidney is regulated in response to chronic alterations in chloride balance. Am J Physiol Renal Physiol 2004;287:F1179-1188.

3 Ambuhl PM, Amemiya, M, Danczkay, M, Lotscher, M, Kaissling, B, Moe, O W, Preisig, P A, Alpern, R J: Chronic metabolic acidosis increases NHE3 protein abundance in rat kidney. Am J Physiol 1996;271:F917-925.

4 Ambuhl PM, Zajicek, H K, Wang, H, Puttaparthi, K, Levi, M: Regulation of renal phosphate transport by acute and chronic metabolic acidosis in the rat. Kidney Int 1998;53:1288-1298.

5 Wang T, Egbert, A L, Jr, Aronson, P S, Giebisch, G: Effect of metabolic acidosis on NaCl transport in the proximal tubule. Am J Physiol 1998;274:F1015-1019.

6 Promeneur D, Kwon TH, Yasui M, Kim GH, Frokiaer J, Knepper MA, Agre P, Nielsen S: Regulation of AQP6 mRNA and protein expression in rats in response to altered acid-base or water balance. Am J Physiol Renal Physiol 2000;279:F1014-1026.

7 Frische S, Kwon, T H, Frokiaer, J, Madsen, K M, Nielsen, S: Regulated expression of pendrin in rat kidney in response to chronic NH_4Cl or $NaHCO_3$ loading. Am J Physiol Renal Physiol 2003;284:F584-593.

8 Stehberger PA, Shmukler BE, Stuart-Tilley AK, Peters LL, Alper SL, Wagner CA: Distal renal tubular acidosis in mice lacking the AE1 (band3) Cl^-/HCO_3^- exchanger (slc4a1). J Am Soc Nephrol 2007;18:1408-1418.

9 Amlal H, Sheriff S, Soleimani M: Upregulation of collecting duct aquaporin-2 by metabolic acidosis: Role of vasopressin. Am J Physiol Cell Physiol 2004;286:C1019-1030.

10 Curthoys NP, Taylor L, Hoffert JD, Knepper MA: Proteomic analysis of the adaptive response of rat renal proximal tubules to metabolic acidosis. Am J Physiol Renal Physiol 2007;292:F140-147.

11 Nijenhuis T, Renkema KY, Hoenderop JG, Bindels RJ: Acid-base status determines the renal expression of Ca^{2+} and Mg^{2+} transport proteins. J Am Soc Nephrol 2006;17:617-626.

12 Windus DW, Cohn DE, Klahr S, Hammerman MR: Glutamine transport in renal basolateral vesicles from dogs with metabolic acidosis. Amer J Physiol Renal Physiol 1984;246:F78-86.

13 Kim GH, Martin SW, Fernandez-Llama P, Masilamani S, Packer RK, Knepper MA: Long-term regulation of renal Na-dependent cotransporters and ENaC: Response to altered acid-base intake. Am J Physiol Renal Physiol 2000;279:F459-467.

14 Tashima Y, Kohda Y, Nonoguchi H, Ikebe M, Machida K, Star RA, Tomita K: Intranephron localization and regulation of the V1a vasopressin receptor during chronic metabolic acidosis and dehydration in rats. Pflugers Arch 2001;442:652-661.

15 Mori T, Inoue T, Nanoguchi H, Nakayama Y, Kohda Y, Machida K, Tomita K: Reduced urinary excretion of aquaporin 2 in metabolic acidosis (abstract). J Am Soc Nephrol 2002:273A.

16 Nonoguchi H, Inoue T, Mori T, Nakayama Y, Kohda Y, Tomita K: Regulation of aquaporin-2 by metabolic acidosis. Amer J Physiol Cell Physiol 2004;287:C824

17 Levine DZ, Nash LA: Effect of chronic NH_4Cl acidosis on proximal tubular H_2O and HCO_3 reabsorption. Amer J Physiol 1973;225:380-384.

18 Ikebe M, Nonoguchi H, Nakayama Y, Tashima Y, Tomita K: Upregulation of the secretory-type $Na^+/K^+/2Cl^-$-cotransporter in the kidney by metabolic acidosis and dehydration in rats. J Am Soc Nephrol 2001;12:423-430.

19 Haldane JB: Experiments on the regulation of the blood's alkalinity: II. J Physiol 1921;55:265-275.

20 Safirstein R, Glassman VP, DiScala VA: Effects of an NH_4Cl-induced metabolic acidosis on salt and water reabsorption in dog kidney. Amer J Physiol 1973;225:805-809.

21 Wang T, Egbert AL, Jr., Aronson PS, Giebisch G: Effect of metabolic acidosis on nacl transport in the proximal tubule. Amer J Physiol Renal Physiol 1998;274:F1015-1019.

22 Faroqui S, Sheriff S, Amlal H: Metabolic acidosis has dual effects on sodium handling by rat kidney. Am J Physiol Renal Physiol 2006;291:F322-331.

23 Nowik M, Lecca MR, Velic A, Rehrauer H, Brandli AW, Wagner CA: Genome-wide gene expression profiling reveals renal genes regulated during metabolic acidosis. Physiol Genom 2008;32:322-334.

24 Ambuhl PM, Zajicek HK, Wang H, Puttaparthi K, Levi M: Regulation of renal phosphate transport by acute and chronic metabolic acidosis in the rat. Kidney Int 1998;53:1288-1298.

25 Moret C, Dave MH, Schulz N, Jiang JX, Verrey F, Wagner CA: Regulation of renal amino acid transporters during metabolic acidosis. Am J Physiol Renal Physiol 2007;292:F555-566.

26 Stehberger PA, Schulz N, Finberg KE, Karet FE, Giebisch G, Lifton RP, Geibel JP, Wagner CA: Localization and regulation of the ATP6V0A4 (a4) vacuolar H^+-ATPase subunit defective in an inherited form of distal renal tubular acidosis. J Am Soc Nephrol 2003;14:3027-3038.

27 Rizzo M, Capasso G, Bleich M, Pica A, Grimaldi D, Bindels RJ, Greger R: Effect of chronic metabolic acidosis on calbindin expression along the rat distal tubule. J Am Soc Nephrol 2000;11:203-210.

28 Mohebbi N, Kovacikova J, Nowik M, Wagner CA: Thyroid hormone deficiency alters expression of acid-base transporters in rat kidney. Am J Physiol Renal Physiol 2007;293:F416-427.

29 Wagner CA, Loffing-Cueni D, Yan Q, Schulz N, Fakitsas P, Carrel M, Wang T, Verrey F, Geibel JP, Giebisch G, Hebert SC, Loffing J: Mouse model of type II Bartters syndrome. II. Altered expression of renal sodium- and water-transporting proteins. Am J Physiol Renal Physiol 2008

30 Dawson TP, Gandhi R, Le Hir M, Kaissling B: Ecto-5'-nucleotidase: Localization in rat kidney by light microscopic histochemical and immunohistochemical methods. J Histochem Cytochem 1989;37:39-47.

31 Fushimi K, Sasaki S, Marumo F: Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. J Biol Chem 1997;272:14800-14804.

32 Kuwahara M, Fushimi K, Terada Y, Bai L, Marumo F, Sasaki S: cAMP-dependent phosphorylation stimulates water permeability of aquaporin-collecting duct water channel protein expressed in Xenopus oocytes. J Biol Chem 1995;270:10384-10387.

33 Nedvetsky PI, Tabor V, Tamma G, Beulshausen S, Skroblin P, Kirschner A, Mutig K, Boltzen M, Petrucci O, Vossenkamper A, Wiesner B, Bachmann S, Rosenthal W, Klussmann E: Reciprocal regulation of aquaporin-2 abundance and degradation by protein kinase a and p38-map kinase. J Am Soc Nephrol 2010

34 Hoffert JD, Nielsen J, Yu MJ, Pisitkun T, Schleicher SM, Nielsen S, Knepper MA: Dynamics of aquaporin-2 serine-261 phosphorylation in response to short-term vasopressin treatment in collecting duct. Am J Physiol Renal Physiol 2007;292:F691-700.

35 Nielsen S, DiGiovanni SR, Christensen EI, Knepper MA, Harris HW: Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. Proc Natl Acad Sci USA 1993;90:11663-11667.

36 Solbu TT, Boulland JL, Zahid W, Lyamouri Bredahl MK, Amiry-Moghaddam M, Storm-Mathisen J, Roberg BA, Chaudhry FA: Induction and targeting of the glutamine transporter SN1 to the basolateral membranes of cortical kidney tubule cells during chronic metabolic acidosis suggest a role in pH regulation. J Am Soc Nephrol 2005;16:869-877.

37 Wagner CA, Finberg KE, Stehberger PA, Lifton RP, Giebisch GH, Aronson PS, Geibel JP: Regulation of the expression of the Cl-/anion exchanger pendrin in mouse kidney by acid-base status. Kidney Int 2002;62:2109-2117.

38 Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park JK, Beck FX, Muller DN, Derer W, Goss J, Ziomber A, Dietsch P, Wagner H, van Rooijen N, Kurtz A, Hilgers KF, Alitalo K, Eckardt KU, Luft FC, Kerjaschki D, Titze J: Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-c-dependent buffering mechanism. Nat Med 2009;15:545-552.

39 Henger A, Tutt, P, Riesen, W F, Hulter, H N, Krapf, R: Acid-base and endocrine effects of aldosterone and angiotensin ii inhibition in metabolic acidosis in human patients. J Lab Clin Med 2000;136:379-389.

40 Schambelan M, Sebastian, A, Katuna, B A, Arteaga, E: Adrenocortical hormone secretory response to chronic NH_4Cl-induced metabolic acidosis. Am J Physiol 1987;252:E454-460.

41 Gyorke ZS, Sulyok, E, Guignard, J P: Ammonium chloride metabolic acidosis and the activity of renin-angiotensin-aldosterone system in children. Eur J Pediatr 1991;150:547-549.

42 Augustinsson O, Johansson, K: Ammonium chloride induced acidosis and aldosterone secretion in the goat. Acta Physiol Scand 1986;128:535-540.

43 Leipziger J, MacGregor GG, Cooper GJ, Xu J, Hebert SC, Giebisch G: PKA site mutations of ROMK2 channels shift the pH dependence to more alkaline values. Am J Physiol Renal Physiol 2000;279:F919-926.

44 Fakler B, Schultz JH, Yang J, Schulte U, Brandle U, Zenner HP, Jan LY, Ruppersberg JP: Identification of a titratable lysine residue that determines sensitivity of kidney potassium channels (ROMK) to intracellular pH. EMBO J 1996;15:4093-4099.

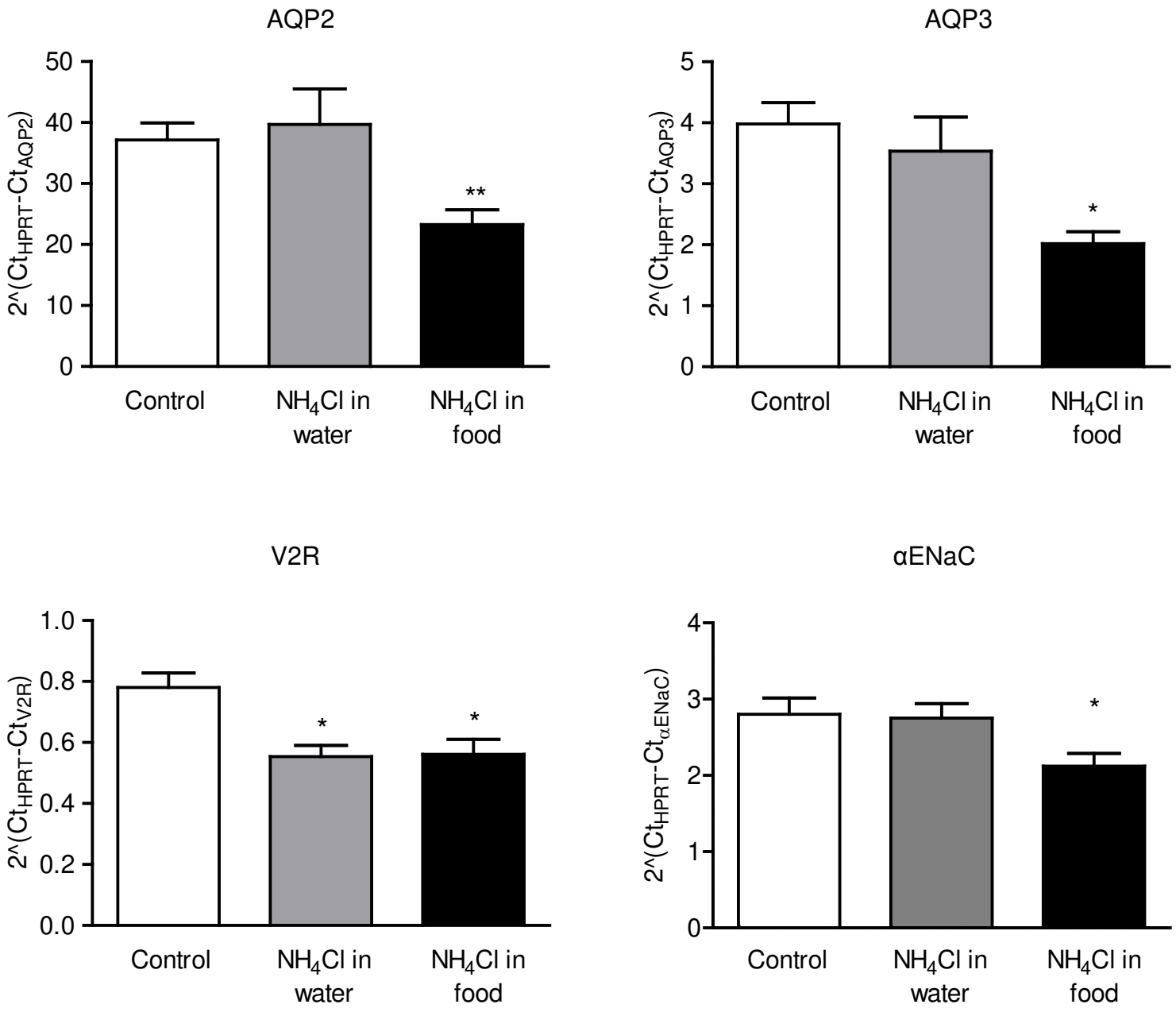
45 Wang W, Li C, Summer S, Falk S, Schrier RW: Interaction between vasopressin and angiotensin ii in vivo and in vitro: Effect on aquaporins and urine concentration. Am J Physiol Renal Physiol 2010;299:F577-584.

46 Kwon TH, Nielsen, J, Masilamani, S, Hager, H, Knepper, M A, Frokiar, J, Nielsen, S: Regulation of collecting duct AQP3 expression: Response to mineralocorticoid. Am J Physiol Renal Physiol 2002;283:F1403-F1421.

47 Renkema KY, Velic A, Dijkman HB, Verkaart S, van der Kemp AW, Nowik M, Timmermans K, Doucet A, Wagner CA, Bindels RJ, Hoenderop JG: The calcium-sensing receptor promotes urinary acidification to prevent nephrolithiasis. J Am Soc Nephrol 2009;20:1705-1713.

48 Sands JM, Naruse M, Baum M, Jo I, Hebert SC, Brown EM, Harris HW: Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. J Clin Invest 1997;99:1399-1405.

Rat kidney


AQP2
2^(Ct_HPRT-Ct_AQP2)
50
40
30
20
10
0
**
Control
NH4Cl in water
NH4Cl in food
AQP3
2^(Ct_HPRT-Ct_AQP3)
5
4
3
2
1
0
*
Control
NH4Cl in water
NH4Cl in food
V2R
2^(Ct_HPRT-Ct_V2R)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Control
NH4Cl in water
NH4Cl in food
αENaC
2^(Ct_HPRT-Ct_αENaC)
4
3
2
1
0
*
Control
NH4Cl in water
NH4Cl in food

Rat kidney

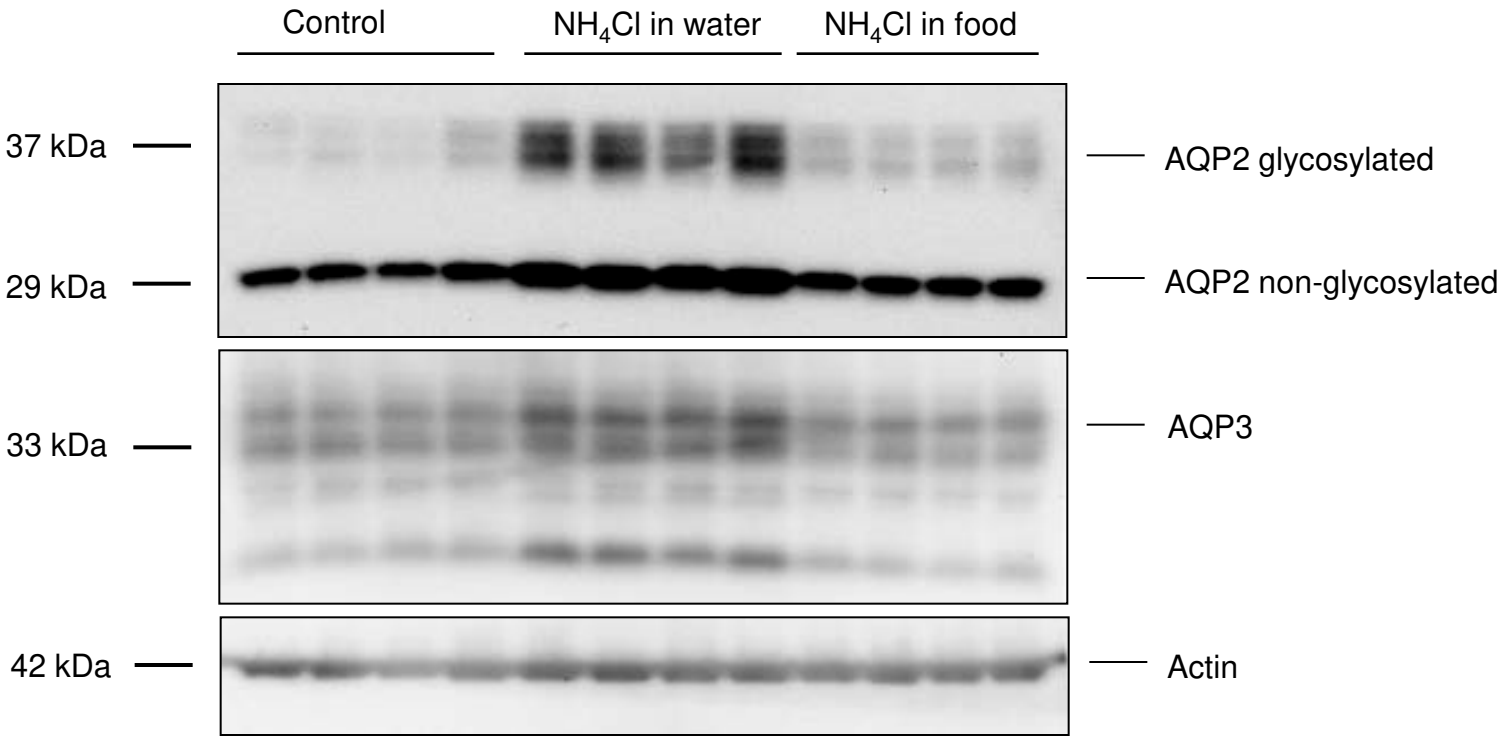
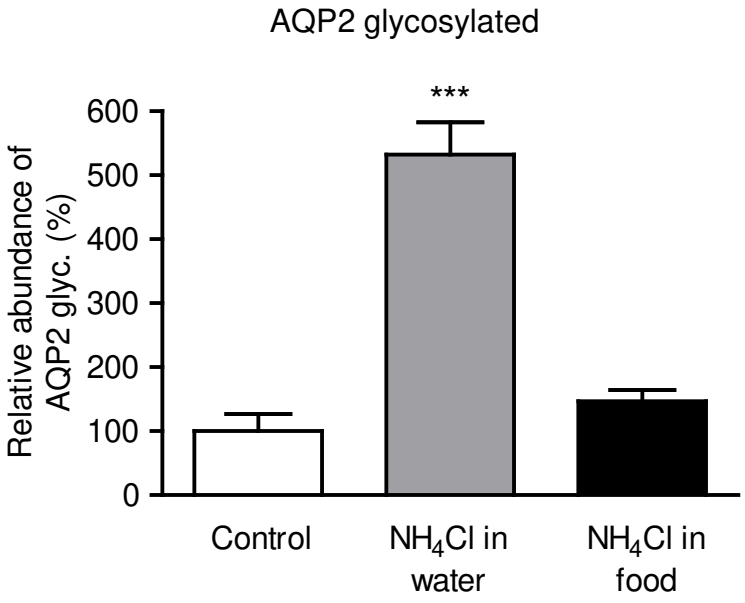
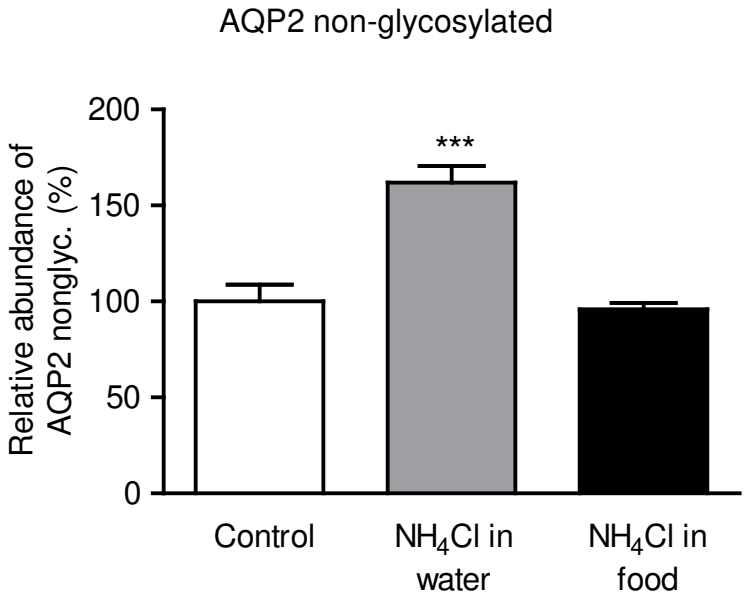

Control
NH4Cl in water
NH4Cl in food
37 kDa
29 kDa
33 kDa
42 kDa
AQP2 glycosylated
AQP2 non-glycosylated
AQP3
Actin

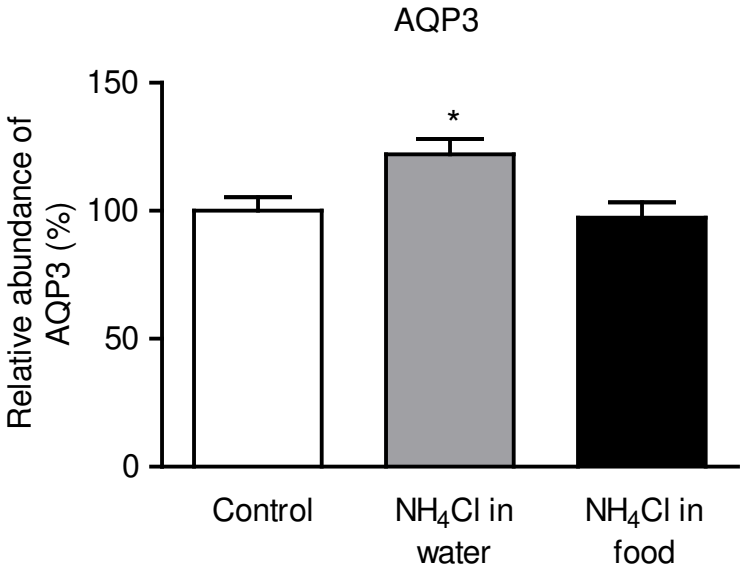
Figure 2 B

Rat kidney

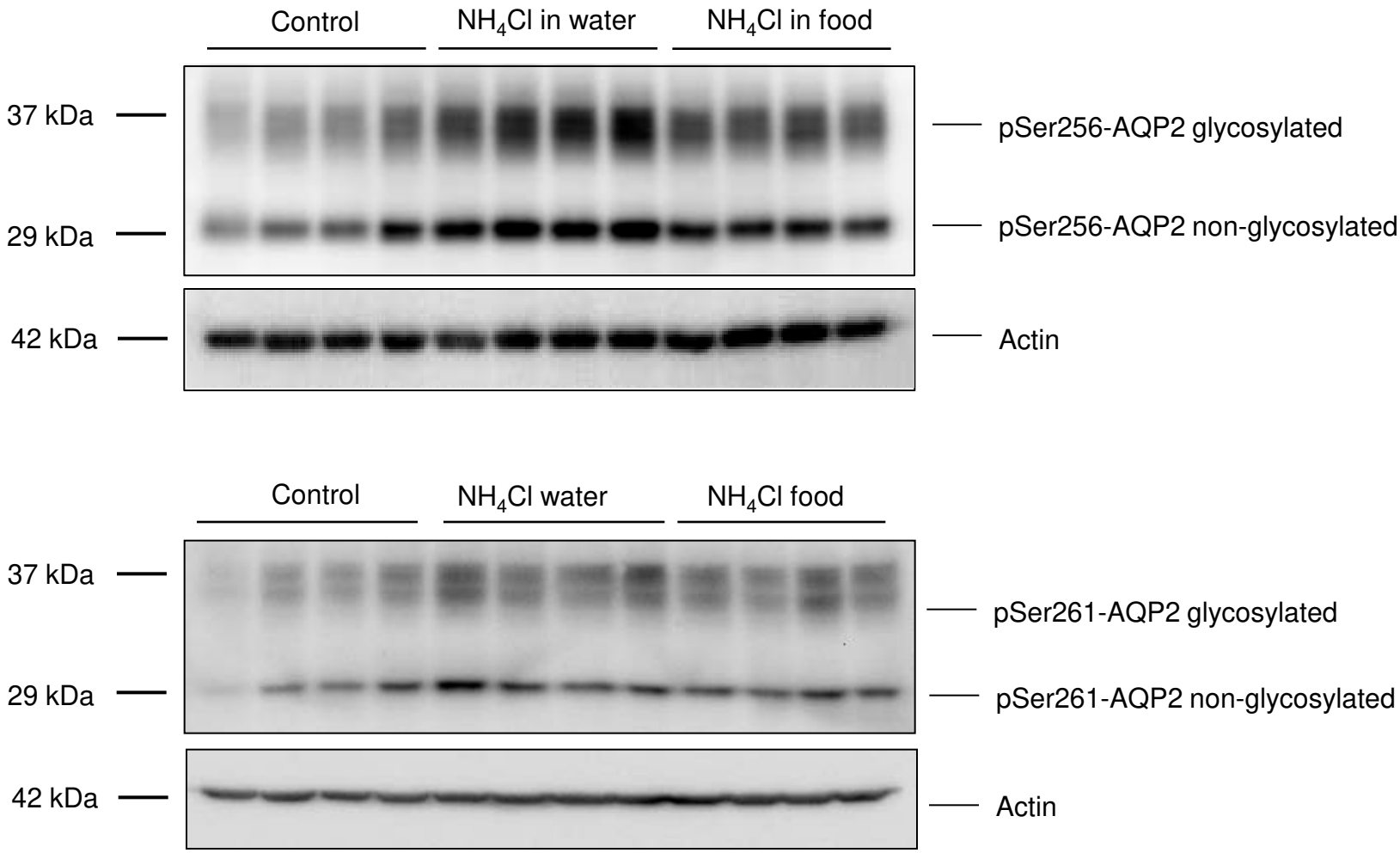
Nowik et al.







Rat kidney


Control
NH4Cl in water
NH4Cl in food
37 kDa
29 kDa
42 kDa
pSer256-AQP2 glycosylated
pSer256-AQP2 non-glycosylated
Actin
Control
NH4Cl water
NH4Cl food
37 kDa
29 kDa
42 kDa
pSer261-AQP2 glycosylated
pSer261-AQP2 non-glycosylated
Actin

Rat kidney

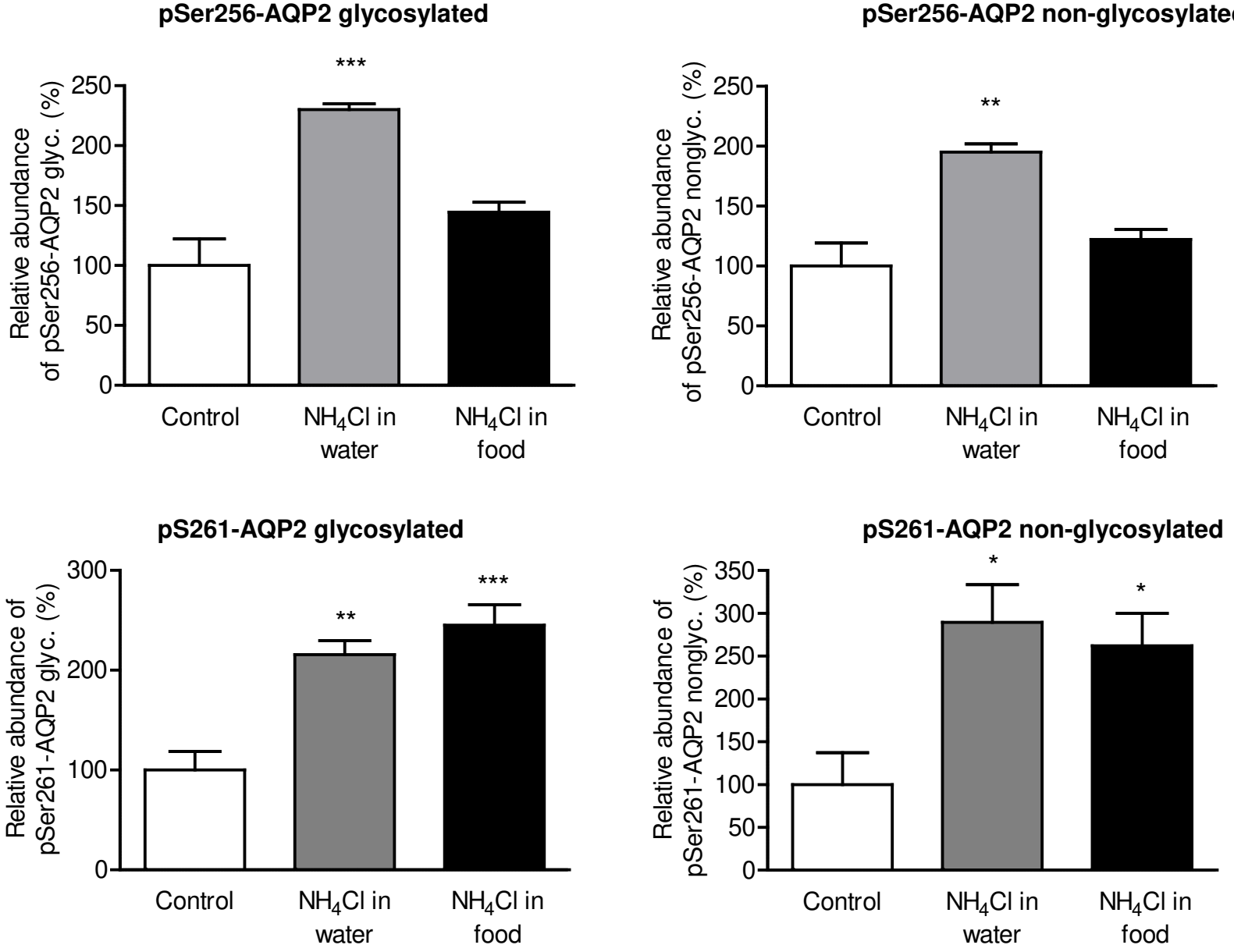

pSer256-AQP2 glycosylated
Relative abundance of pSer256-AQP2 glyc. (%)
250
200
150
100
50
0
***
Control
NH4Cl in water
NH4Cl in food
pSer256-AQP2 non-glycosylated
Relative abundance of pSer256-AQP2 nonglyc. (%)
250
200
150
100
50
0
**
Control
NH4Cl in water
NH4Cl in food
pS261-AQP2 glycosylated
Relative abundance of pSer261-AQP2 glyc. (%)
300
200
100
0
**
***
Control
NH4Cl in water
NH4Cl in food
pS261-AQP2 non-glycosylated
Relative abundance of pSer261-AQP2 nonglyc. (%)
350
300
250
200
150
100
50
0
*
*
Control
NH4Cl in water
NH4Cl in food

Figure 4A

Rat kidney

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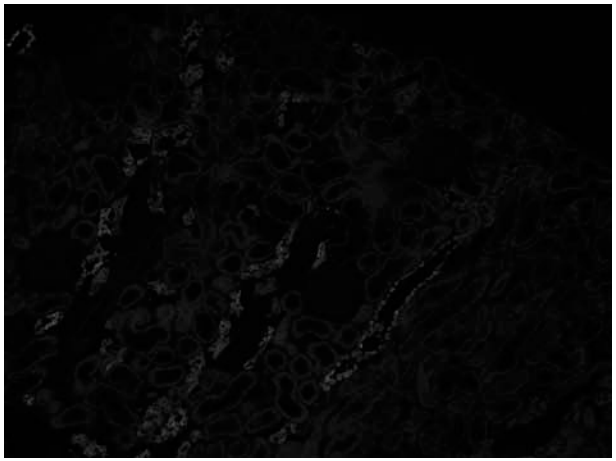
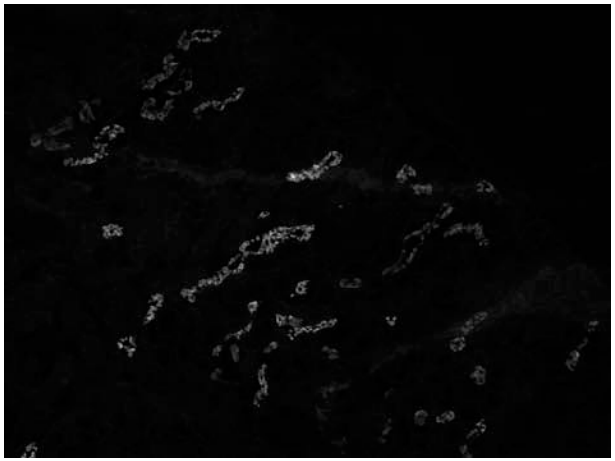
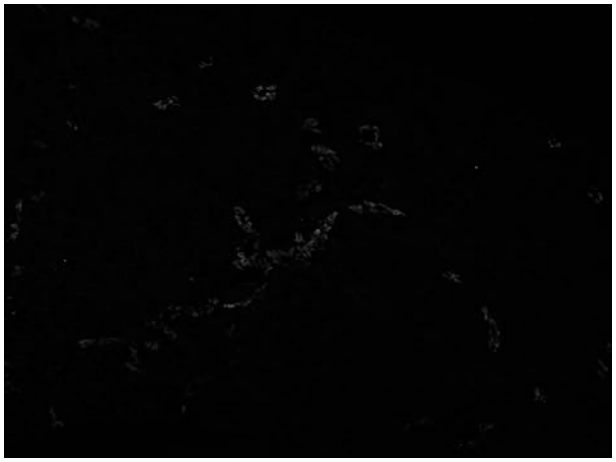
Cortex



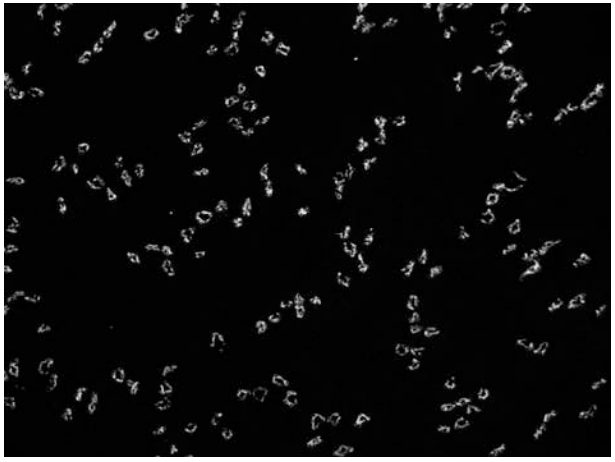




Control

NH_4Cl in water

NH_4Cl in food

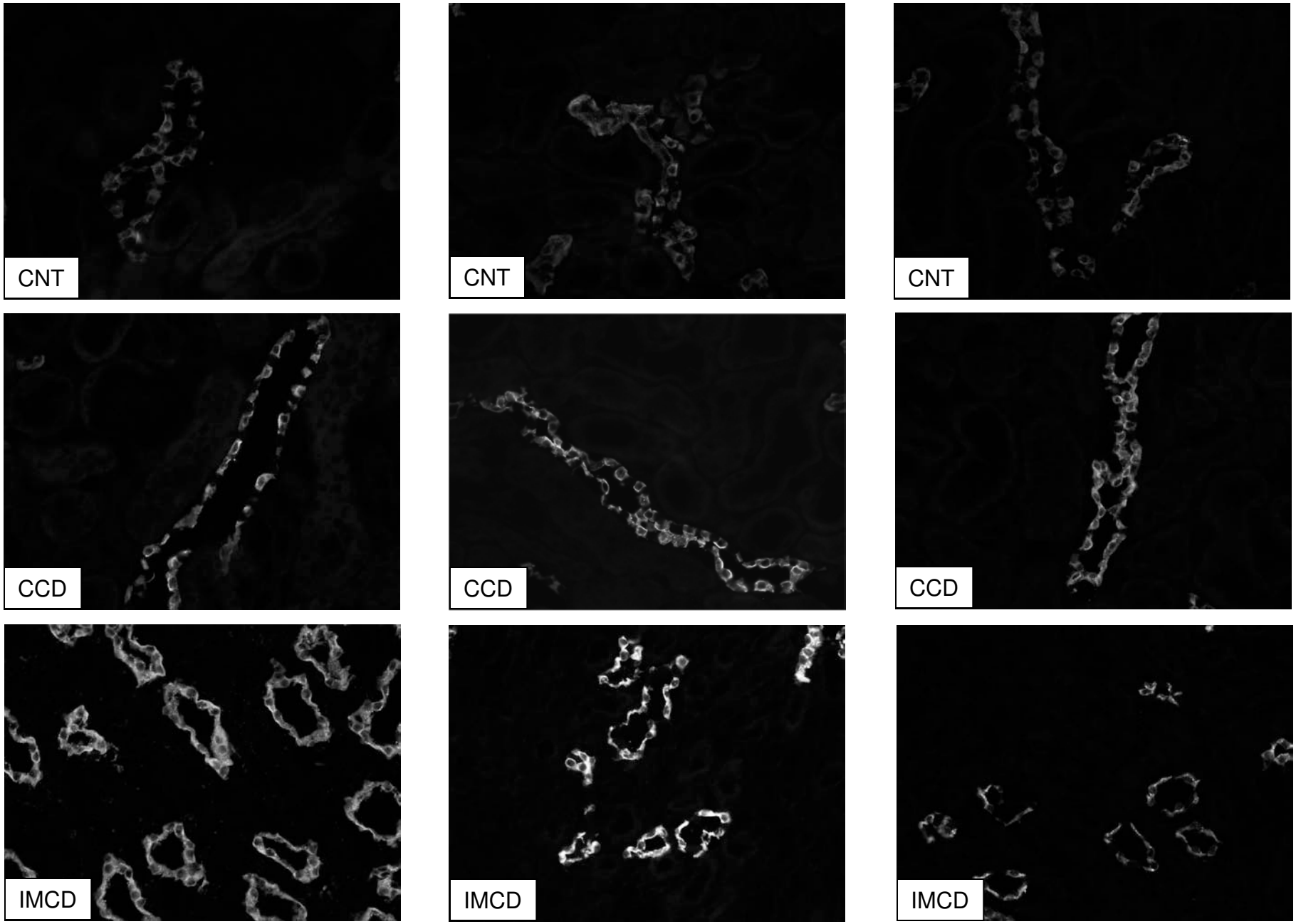


Inner medulla

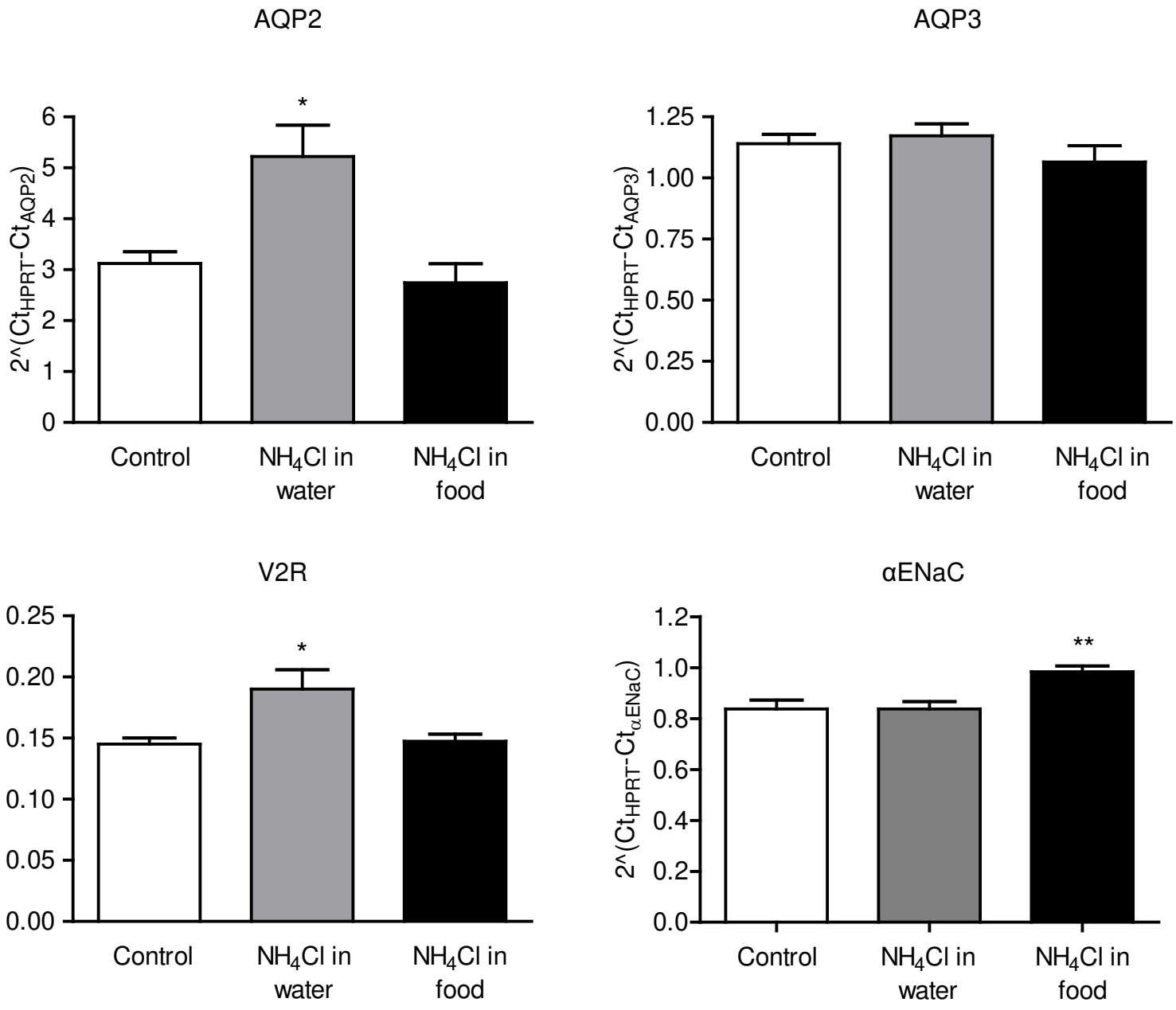
Figure 4B

Nowik et al.

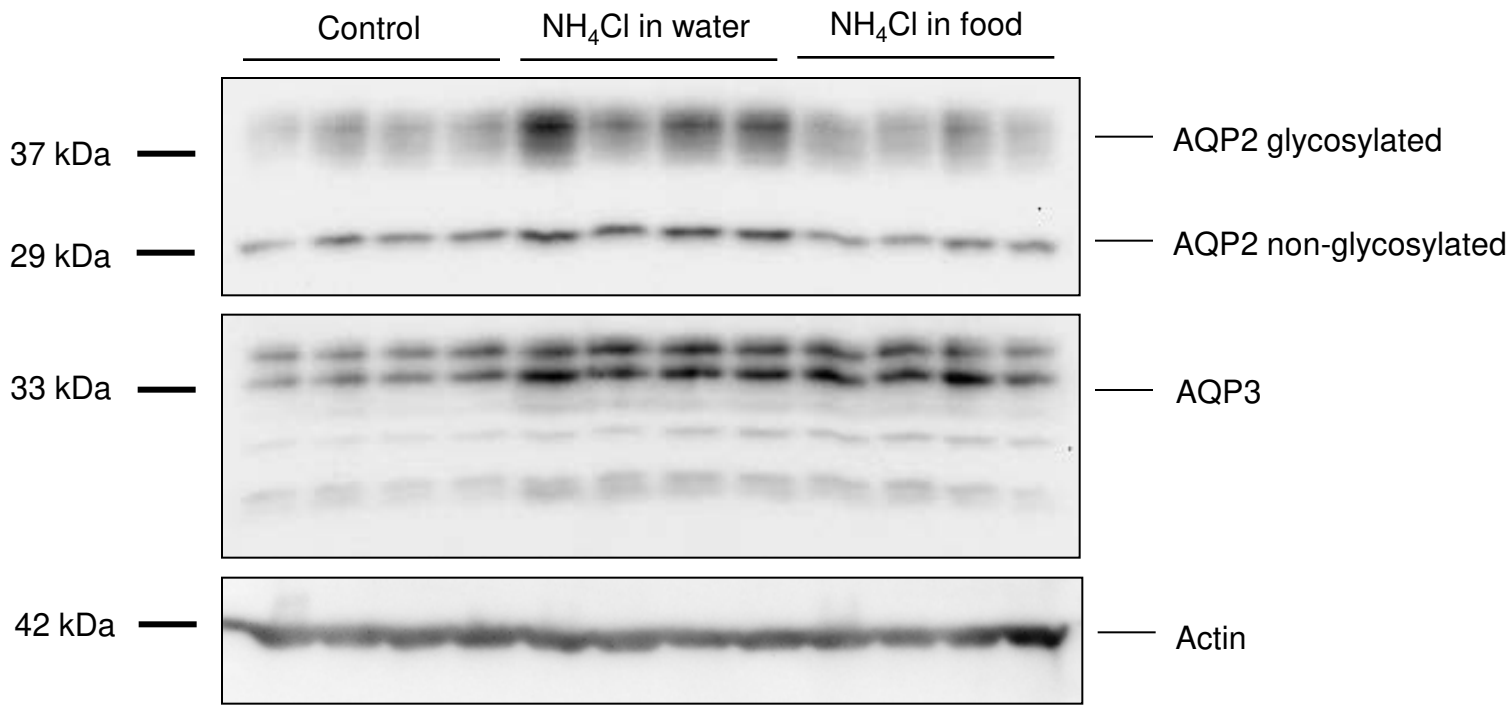
Rat kidney



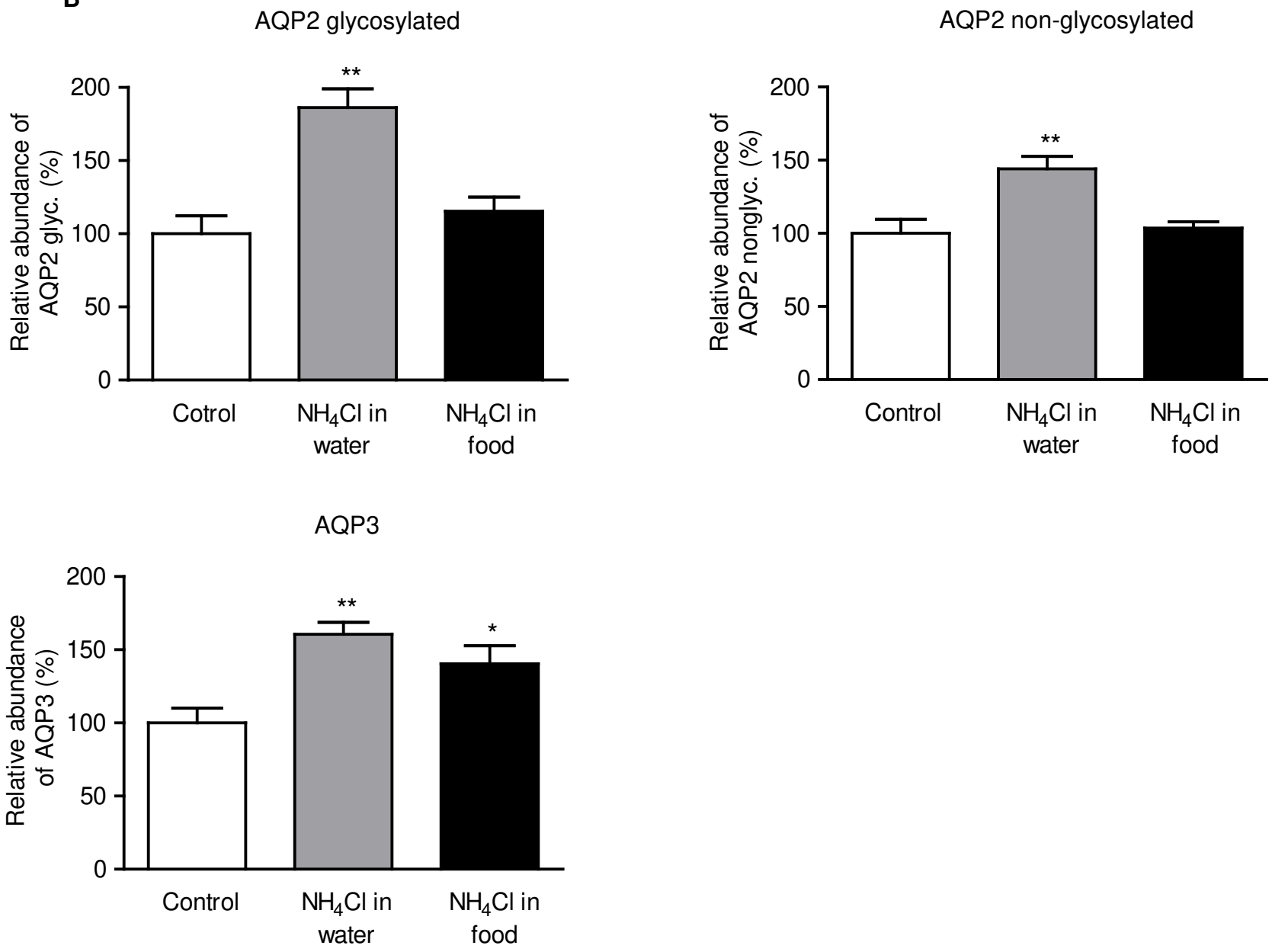
Mouse kidney



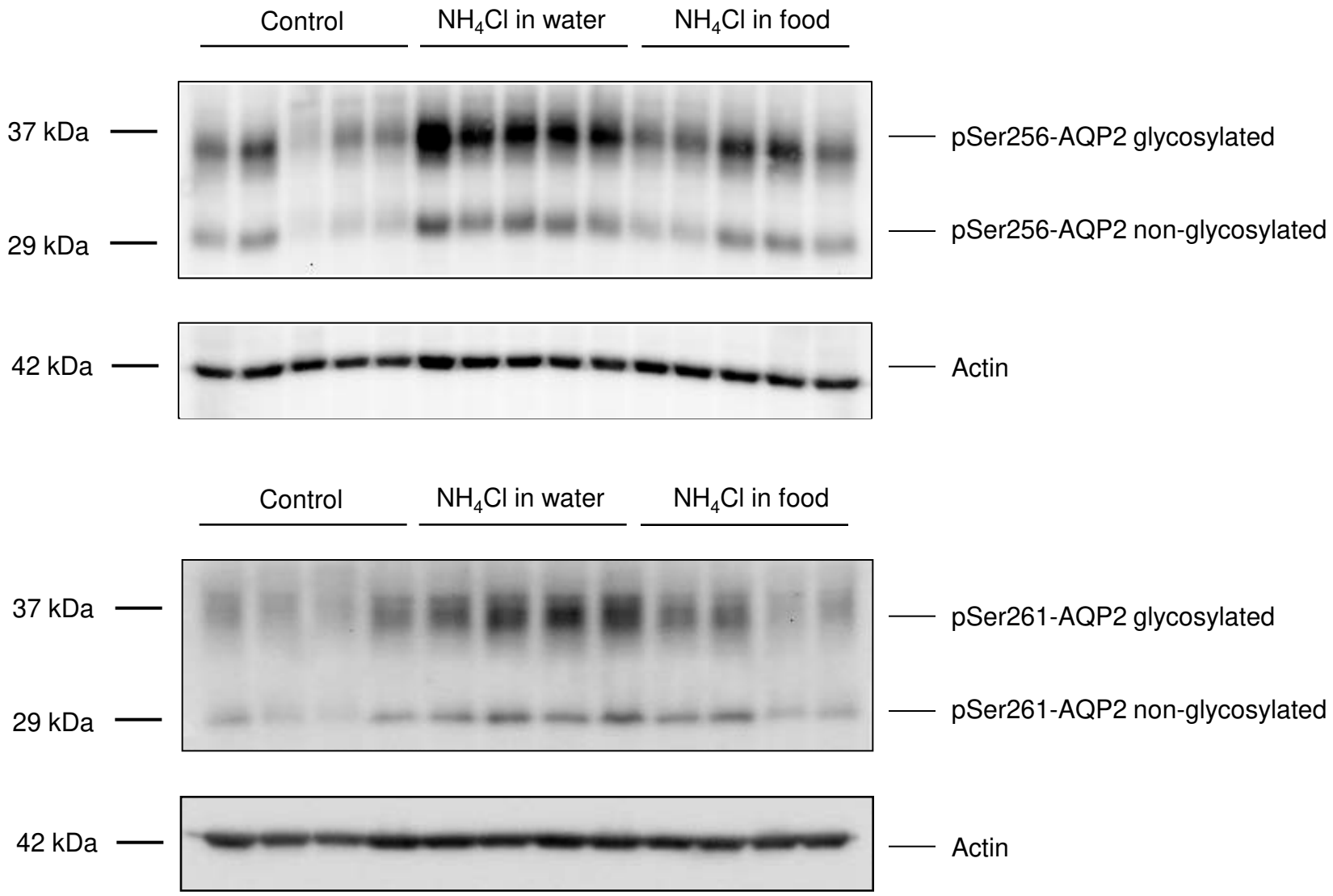
Mouse kidney



B


AQP2 glycosylated
Relative abundance of AQP2 glyc. (%)
200
150
100
50
0
**
Cotrol
NH4Cl in water
NH4Cl in food
AQP2 non-glycosylated
Relative abundance of AQP2 nonglyc. (%)
200
150
100
50
0
**
Control
NH4Cl in water
NH4Cl in food
AQP3
Relative abundance of AQP3 (%)
200
150
100
50
0
**
*
Control
NH4Cl in water
NH4Cl in food

Mouse kidney



Mouse kidney

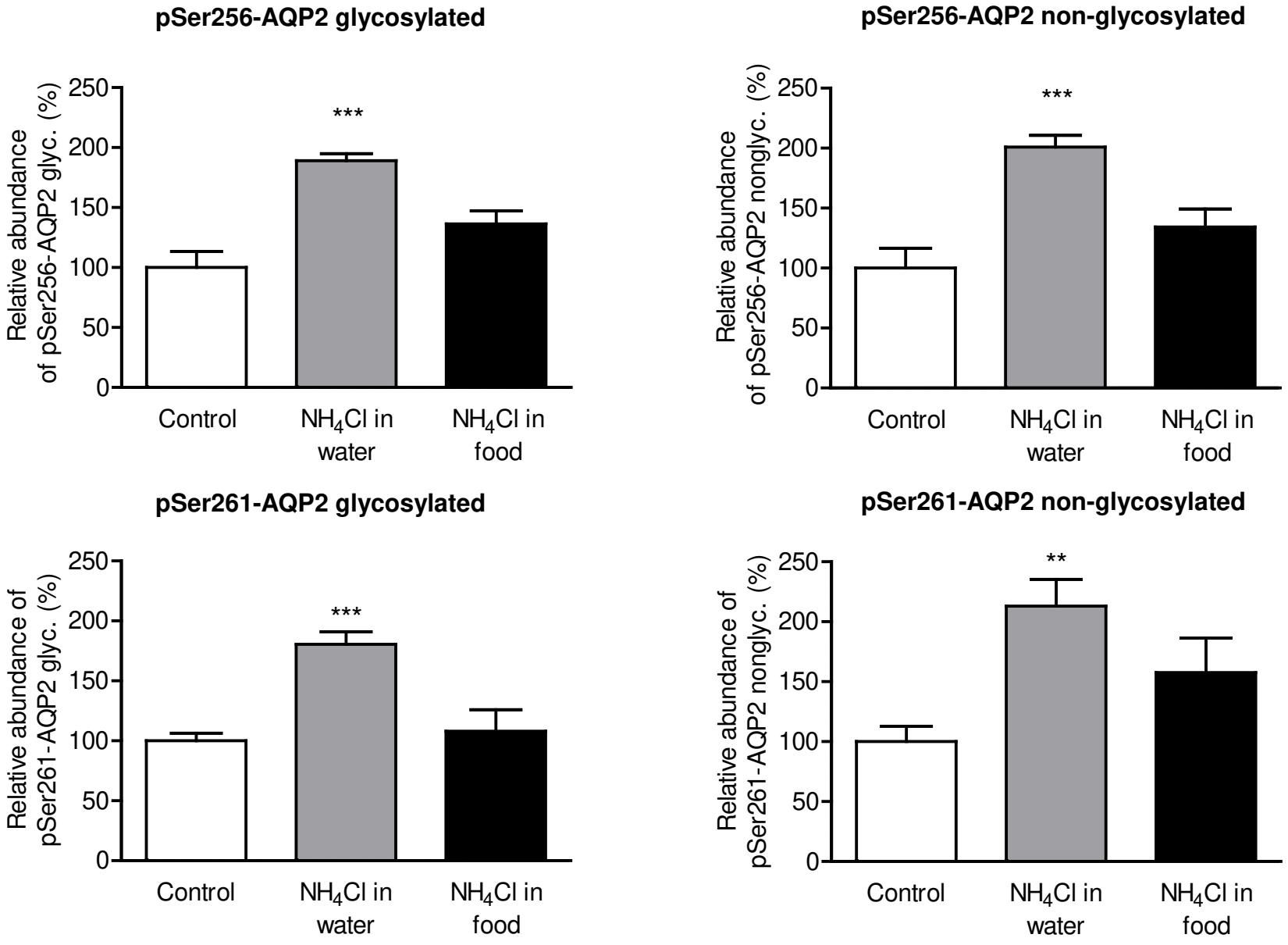
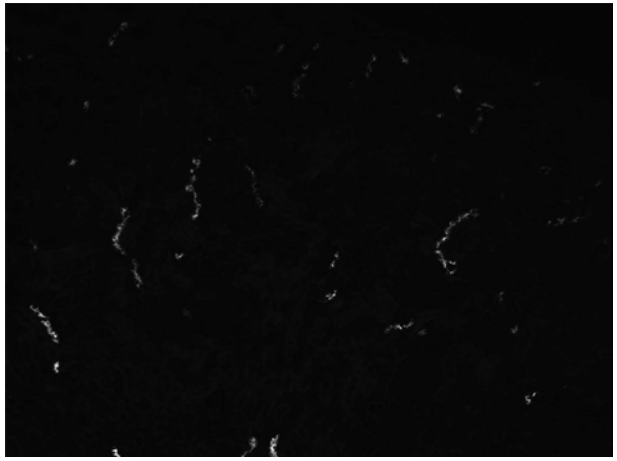


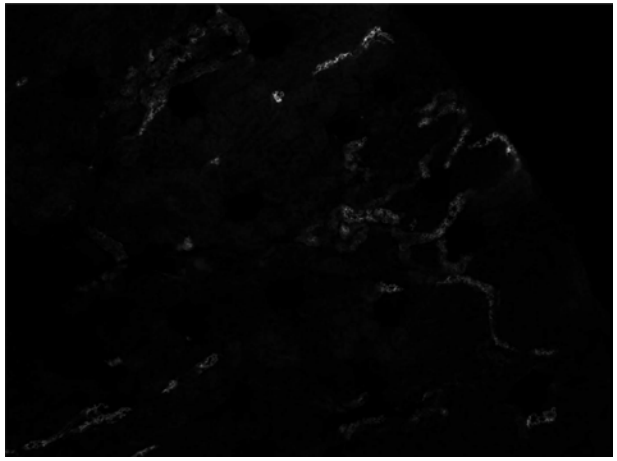
Figure 8A

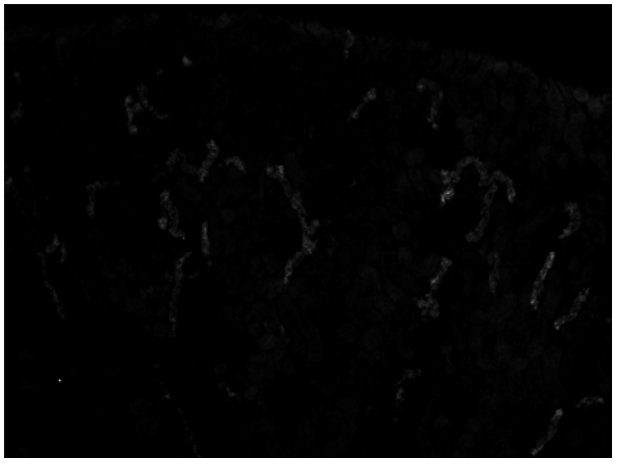
Mouse kidney

Nowik et al.

Cortex



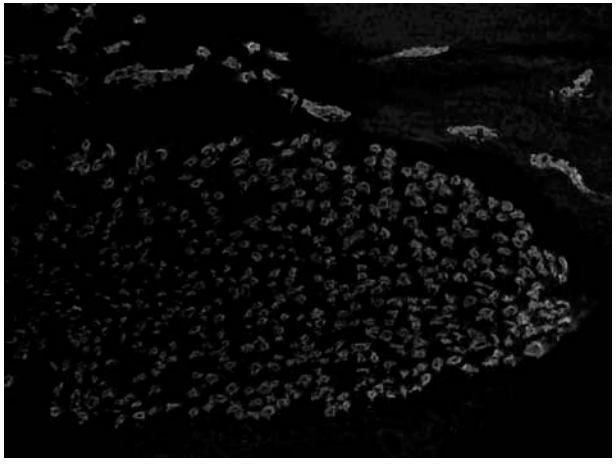


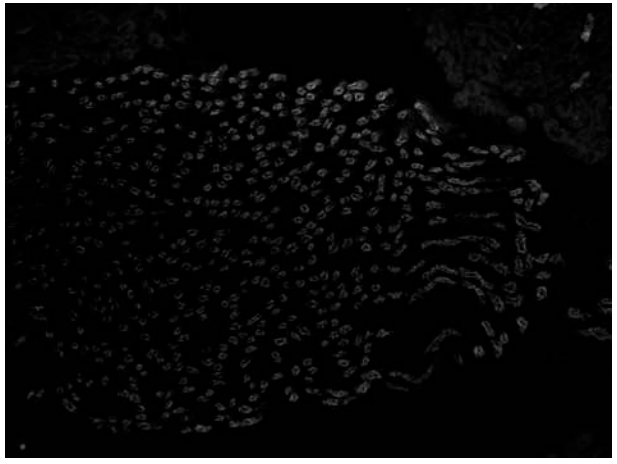


Control

NH_4Cl in water

NH_4Cl in food





Inner medulla

Figure 8B

Mouse kidney

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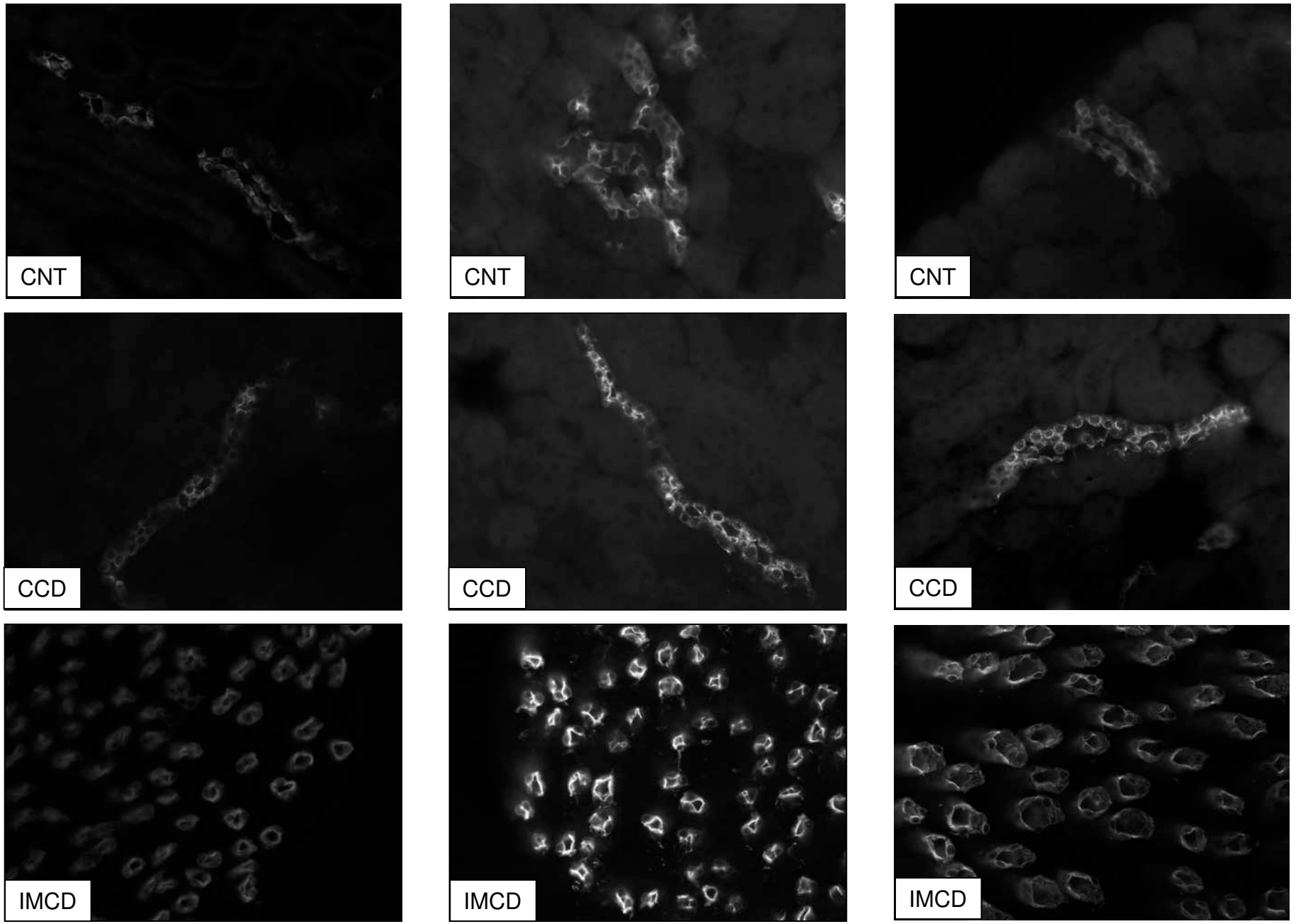

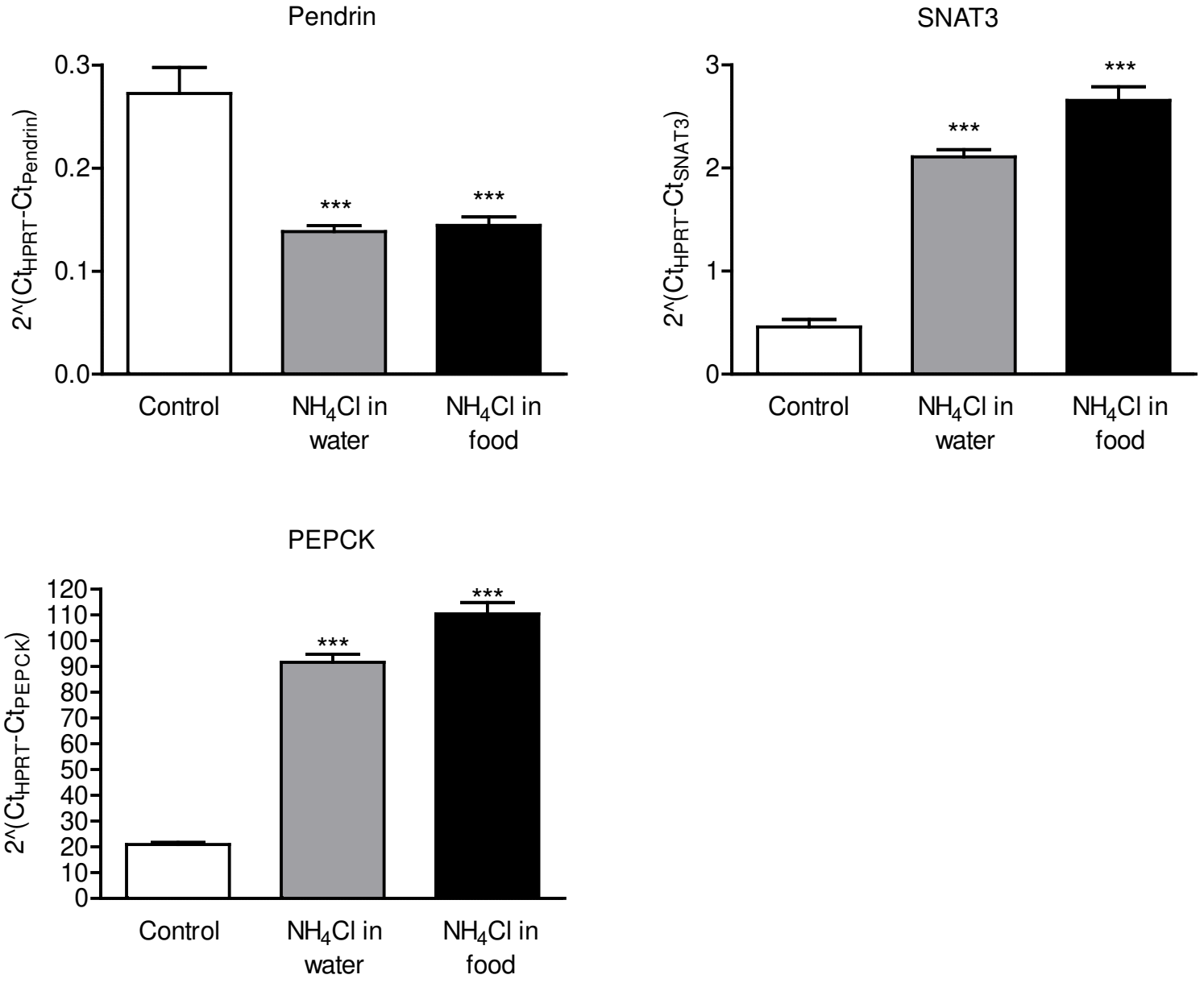
Pendrin
2^(Ct$_{HPRT}$-Ct$_{Pendrin}$)
0.3
0.2
0.1
0.0
***
***
Control
NH$_4$Cl in water
NH$_4$Cl in food
SNAT3
2^(Ct$_{HPRT}$-Ct$_{SNAT3}$)
3
2
1
0
***
***
Control
NH$_4$Cl in water
NH$_4$Cl in food
PEPCK
2^(Ct$_{HPRT}$-Ct$_{PEPCK}$)
120
110
100
90
80
70
60
50
40
30
20
10
0
***
***
Control
NH$_4$Cl in water
NH$_4$Cl in food